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CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

VI. THE ISOLATION FROM DISEASED TURKISH TOBACCO PLANTS OF A CRYSTALLINE PROTEIN POSSESSING THE PROPERTIES OF TOBACCO-MOSAIC VIRUS[1]

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During the past few years rather extensive chemical studies have been made on juices from tobacco plants infected with tobacco-mosaic virus. During the course of these investigations it was found, in experiments with pepsin (15) and with certain protein precipitants (16), that this virus acts as a protein. As a result of chemical work, Vinson and Petre (18, 19) and other workers (1, 4) believed tobacco-mosaic virus to be a non-living chemical agent, although certain investigators have regarded it and other viruses as living agents (3, 5). Rivers (11) and Burnet and Andrewes (3) have published reviews and bibliographies relative to the nature of viruses. The evidence in support of a belief in the protein nature of tobacco-mosaic virus seemed sufficiently strong to warrant a further study employing chemical methods. This study resulted in the isolation of a crystalline protein having the properties of tobacco-mosaic virus (17). The present paper presents in detail the methods of concentration and purification and the general properties of this protein.

EXPERIMENTAL

Preparation of Crude Globulin

The active crystalline protein has been isolated from several different lots of infected plants, some of which were grown in the greenhouse at different times during the summer and winter and some of which were grown outside in the field during the summer. The description and data included in the following paragraphs are for infected plants grown in a field during the past summer.

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

Small Turkish tobacco plants about 4 to 6 inches high were planted in a field during the week of May 20, 1935. The plants were inoculated with the ordinary strain of tobacco-mosaic virus (Johnson's tobacco virus 1) on June 13, 1935, by rubbing one leaf of each plant once with a bandage gauze pad moistened with a preparation consisting of infectious juice diluted with 100 parts of water. When the plants had reached a stage of maturity indicated by the beginning of flower-bud formation, which was during the week of July 15, 1935, they were cut off about 6 to 10 inches above the ground, placed in large burlap bags, and immediately transferred to a room held at a temperature of –8° C. The stalks in the ground sprouted new shoots and about a month later a second crop of infected plants was cut and stored. A third crop was obtained after about another month. The second crop gave the largest harvest and the third crop the smallest.

As soon as the plants were completely frozen they were removed, a bag at a time, and immediately, while still frozen, put through a large power meat grinder.[2] It was found much easier to grind the frozen material than either the fresh material or the frozen and thawed material. The macerated pulp was caught in 5-gallon tinned dairy pails.[3] To each pail of pulp was then added 750 cc. of a solution prepared by making 20 lb. of granular U. S. P. disodium phosphate up to 15 liters with hot tap water. It was found necessary to have the pail, in which this solution was prepared, immersed in hot water for a few minutes in order to get all of the disodium phosphate into solution. The pulp and the disodium phosphate solution were thoroughly mixed by hand and then allowed to stand overnight in order to allow the frozen pulp to thaw thoroughly.

The pulp was then transferred to large bags made of 4 layers of bandage gauze and holding one pail of pulp each. These bags were suspended from a hook and after the excess liquid had drained off some additional liquid was obtained by turning and pressing the bags by hand. The bags were then subjected to pressure in a fruit press[4] of sufficient size to hold one bag, and additional liquid was pressed out. All of the liquid obtained from the pulp in this first extraction was combined and placed in 5-gallon tinned pails. The fruit press, which was found preferable to a small hydraulic press, brought the yield of juice up to about 70 per cent of the volume of the pulp. The pails were covered and placed in a cool room or in a low-temperature room held at –8° C. The press cakes of pulp from the first extraction, each representing one pail of the original macerated material, were immediately thoroughly mixed with a volume of cold tap water equal to that of the first extract. Then 750 cc. of the disodium phosphate solution was added to each

[2] Model No. 432SP obtained from The Enterprise Mfg. Co., Philadelphia, Pa.

[3] No. 5550 obtained from The Atlantic Stamping Co., Rochester, N. Y.

[4] Similar to No. 20 of the Baccellieri Mfg. Co., Berlin, N. J.

pail of pulp mixture. The covered pails were allowed to stand overnight in the low-temperature room. The next morning they were transferred to bags and a second extract obtained by pressing by hand and by means of the fruit press. The second extract was allowed to stand in the low-temperature room overnight and the press cakes from the second extract were discarded. The procedure just described, involving 2 extractions and the use of disodium phosphate, which was used to decrease the hydrogen-ion concentration of the extracts to between pH 6 and 7, was found to yield from 2 to 10 times more virus than any of the other methods used.

After standing overnight, the first extract was decanted from a small amount of green-colored precipitate, about 200 gm. of celite (Hyflo Super-cel[5]) was added to each pail of extract, and, after stirring, the mixture was filtered through a ¼-inch layer of celite on a large Büchner funnel. In order to facilitate filtration, it was found advisable to remove occasionally the uppermost layer of the filter cake from the Büchner funnel. The celite thus removed was not discarded but was added to the pails of extract to be filtered. It was found possible to use the same celite several times before it slowed the filtration sufficiently to necessitate discarding it. The addition of celite to the freshly pressed juice permitted rapid filtration of the juice through the layer of celite on the Büchner funnel. This operation gave a sparkling clear brownish filtrate. Table 1 records the data concerning the total solids, ash, total nitrogen, and protein nitrogen of juice from the first harvest before and after filtration through celite and the same data for the

TABLE 1.—*Total solids, ash, total nitrogen and protein-nitrogen determinations on dilute disodium phosphate extracts from frozen, macerated, infected Turkish tobacco plants before and after filtration through celite*[a]

	Mg. total solids per cc.[b]	Mg. ash per cc.[b]	Mg. total N per cc.[b]	Mg. protein-N per cc.[b]	Mg. total N per cc.[c]	Mg. protein-N per cc.[c]
1st extract before filtration	47.1	19.3	2.96	1.07	2.65	1.24
1st extract after filtration	43.9	17.9	2.96	0.87	2.65	1.18
2nd extract before filtration	34.6	20.8	2.08	0.70	2.20	0.47
2nd extract after filtration	32.6	19.6	1.78	0.45	2.02	0.42

[a] Infectivity data are given in table 2.

[b] Data from first crop of plants.

[c] Data from second crop of plants.

[5] All celite preparations were obtained from Johns-Manville, 22 East 40th Street, New York, N. Y.

second extract of the pulp. Total nitrogen and protein-nitrogen data for juice from the second harvest are included. The table shows that there was only a slight loss of protein on filtration through celite. There was also a slight reduction in total solids, total nitrogen and ash in most instances. Although the second extracts contained almost $\frac{1}{2}$ as much protein as the first extracts, it was found that a third extraction gave only about $\frac{1}{10}$ as much protein as the first extraction. Therefore, the macerated material was subjected to only 2 extractions. The infectiousness of the first and second extracts before and after filtration was determined by means of inoculations to leaves of *Nicotiana glutinosa* L., using the half-leaf method (6, 13, 14). The results, given in table 2, show that there is no great difference in the

TABLE 2.—*Infectivity tests on 2 dilute disodium phosphate extracts from frozen macerated infected Turkish tobacco plants before and after filtration through celite*[a]

Dilution →	1	1–10	1–100	1–1000	1–10,000	1–100,000	1–1,000,000
1st extract before filtration	29.7[b]	30.0	18.3	8.9	0.6	0.1	0.0
1st extract after filtration	32.4[c]	36.0	45.0	10.2	0.5	0.0	0.0
2nd extract before filtration	52.5	24.5	21.0	5.8	2.1	0.0	0.0
2nd extract after filtration	45.7	19.8	22.4	10.0	1.9	0.1	0.0
1st extract before filtration	40.0	23.4	10.7	10.0	0.2	1.1	0.0
2nd extract before filtration	59.6	31.8	12.5	10.3	0.3	0.6	0.0
1st extract after filtration	34.7	47.1	18.2	14.6	0.7	0.1	0.0
2nd extract after filtration	61.3	52.7	16.3	7.2	0.8	0.2	0.0

[a] Analytical data are given in table 1.

[b] Numbers represent the average number of lesions per half-leaf obtained on the left halves of 5 leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution. Dilutions were made with distilled water.

[c] Numbers represent the average number of lesions per half-leaf obtained on the right halves of the same leaves on inoculation with the designated preparation and dilution. Dilutions were made with distilled water.

infectivity of these 4 preparations. Thus the protein content and the infectivity of the extracts are about the same before and after filtration through celite.

To each pail containing about 15 liters of 1st-extraction filtrate was added about 200 cc. of 3M H_2SO_4, with vigorous stirring, in order to adjust the hydrogen-ion concentration to about pH 5. Smaller amounts of 3M H_2SO_4 (from 100 to 140 cc.) were used to adjust the 2nd-extraction filtrate to the same hydrogen-ion concentration. The globulin fraction was precipitated by adding, with stirring, 3 kg. of ammonium sulphate to each pail (9, 18), and the mixture was then placed in a cool room overnight. The mixtures were then filtered through folded filter paper, using 10-inch glass funnels and 50 cm. C. S. and S. No. 1450½ paper. Much more color was lost in the filtrate when this type of filtration was used than when the filtration was carried out on a Büchner funnel with suction, using either paper or paper and celite. About one pail could be filtered through one paper during 24 hours. During this and all subsequent operations the activity of the precipitates and of the filtrates was repeatedly checked by means of infectivity tests on leaves of *Nicotiana glutinosa*. The dark-colored filtrates were found to contain little or no virus and were discarded. The dark brown paste-like precipitates were removed from the filter paper by means of a spatula, combined, and placed on large Büchner funnels in order to remove as much of the colored fluid as possible. The papers from which most of the globulin had been removed were collected, extracted with dilute disodium phosphate solution, and the extract adjusted to about pH 5 and to about 0.4 saturation with ammonium sulphate. The precipitate was removed by filtration and added to the main portion.

After being subjected to filtration with suction on the Büchner funnel for a day, the dark brown globulin fraction was divided into lots of from 200 to 300 gm. Each lot was suspended in 15 liters of water in a pail to which 0.5 kg. disodium phosphate was added. The hydrogen-ion concentration was adjusted to about pH 8 by the addition, with stirring, of about 300 cc. of 2N NaOH per pail of mixture. To each pail was then added 200 gm. of Hyflo Standard-cel, with stirring, and the mixture was then filtered with suction through a ¼-inch layer of the same material on a large Büchner funnel. Data on the protein content of many different preparations before and after filtration through either Hyflo Super-cel or Hyflo Standard-cel indicated that from 5 to 30 per cent, with an average of about 20 per cent, of the protein remained on the filter cake. Although it was found possible to recover practically all of this protein by 1 or 2 extractions of the celite filter cake, this was not done routinely because the amount of the recovered protein did not warrant the added labor involved in the recovery.

Following the filtration through celite, the filtrate was adjusted to pH 5 by the addition of about 150 cc. of 3M H_2SO_4. Three kg. of ammonium sulphate were then added to each pail of filtrate. After standing in a cool room overnight, the precipitate was collected by filtration using folded C. S.

and S. No. 1450½ paper. This procedure, in which the globulin fraction was taken into solution, filtered through celite and again precipitated, was repeated from 2 to 4 times in order to obtain a filtrate from the precipitated globulin that was practically colorless. The precipitate was still dark brown, despite the fact that much color had been lost in the filtrates from the precipitated and reprecipitated globulin. Table 3 records the tests for

TABLE 3.—*Infectivity tests on once-precipitated globulin from first extract, once-precipitated globulin from second extract, and on 5-times-precipitated globulin*

Dilution →		$1–10^3$	$1–10^4$	$1–10^5$	$1–10^6$	$1–10^7$	$1–10^8$	$1–10^9$
Once-precipitated globulin. 1st extract.	P. vulgaris	340.5[a]	236.0	114.0	64.5	19.2	3.9	1.1
	N. glutinosa	144.0[a]	84.3	60.6	45.5	10.2	3.2	0.2
Once-precipitated globulin. 2nd extract.	P. vulgaris	385.0	382.5	59.6	60.0	3.2	2.2	1.3
	N. glutinosa	182.0	175.0	134.0	33.0	8.0	0.8	1.8
5-times-precipitated globulin from mixture of extracts.	N. glutinosa	275.5	132.5	133.5	26.7	15.8	8.9	2.6

[a] Numbers represent the average number of lesions per leaf obtained on 10 or more leaves of *Phaseolus vulgaris* or on 5 or more leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution. Dilutions were made with 0.1 M phosphate at pH 7. A dilution of $1–10^3$ denotes 0.001 gm. of protein per cc., $1–10^4$ denotes 0.0001 gm. of protein per cc., etc.

infectivity on 3 separate globulin preparations. The dilutions are based on the actual amount of globulin present per cc., viz., a dilution of $1–10^3$ denotes 0.001 gm. of protein per cc., $1–10^4$ denotes 0.0001 gm. of protein per cc., etc. It may be seen that the infectivity of the 3 different crude globulin preparations is about the same. The dark brown globulin fraction, prepared by precipitation and reprecipitation with ammonium sulphate until there was no further loss of color in the filtrate, was found to be infectious at a dilution of 10^{-9} gm. per cc. It is referred to as crude globulin and is the starting material that was used for purification.

During the past summer a total of about 4000 kg. of infected fresh Turkish tobacco plants have been processed according to the scheme outlined above. The 4000 kg. of fresh plant material yielded about 5000 liters of extract, in 2 extractions, which contained about 22 kg. of protein before filtration through Hyflo Standard-cel and about 18 kg. of protein after filtration. From this 18 kg. of protein about 14 kg. of once-precipitated globulin was obtained. On reprecipitation a yield of about 80 per cent or 11 kg. of crude globulin was obtained. The globulin fraction thus isolated amounts to about 5.5 per cent of the total solids present in the original extract from

the plant material, or about 0.2 per cent by weight of the extract itself. About $\frac{1}{3}$ of the total nitrogen present in the extract is protein-nitrogen, and of this total about $\frac{1}{2}$ was isolated in the form of the crude globulin-nitrogen.

Purification and Crystallization of the Globulin

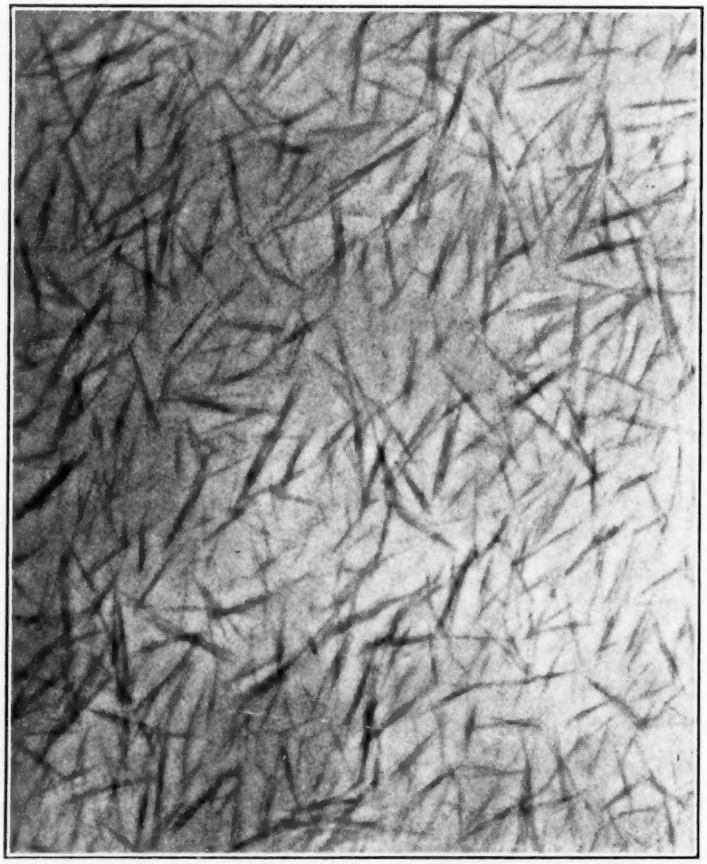
A solution containing 1.2 per cent by weight of crude globulin was adjusted to pH 8.8 with 2N NaOH. Then to 11 different 200 cc. portions of this solution were added varying amounts of a solution containing 200 gm. of lead subacetate per liter. The preparations were then adjusted to about pH 8.8 with 2N NaOH and each filtered through separate Büchner funnels containing a $\frac{1}{4}$-inch layer of Hyflo Standard-cel. The 11 different filtrates were then adjusted to about pH 7 and protein-nitrogen determinations were made on each sample. The filtrates from preparations to which 1, 2, and 4 cc. of lead subacetate solution had been added were still very dark, but the one to which 6 cc. had been added was definitely lighter in color, and the one to which 8 cc. had been added was yellow and opalescent in appearance. Filtrates from preparations to which 10 or more cc. of lead subacetate solution had been added had an opalescent appearance and varied in color from light yellow to colorless. However, it may be seen from table 4 that, when

TABLE 4.—*Effect of lead subacetate treatment on the protein content and color of a 1.2 per cent solution of active globulin*

Number of cc. of lead subacetate solution added to 200 cc. of a 1.2% protein solution	Total protein in filtrate from lead subacetate precipitate	Per cent protein recovered in filtrate	Color of filtrate
1	2.33	97	Dark brown
2	2.26	94	" "
4	2.29	95	" "
6	1.66	69	Dark yellow
8	1.16	50	Yellow
10	0.35	15	Light yellow
13	0.13	5	Practically colorless
16	0.04	2	" "
20	0.02	1	" "
25	0.03	2	" "
30	0.04	2	" "

10 or more cc. of lead subacetate solution were added to 200 cc. of a 1.2 per cent solution of globulin, only from 1 to 15 per cent of the globulin was recovered in the filtrate. When 6 or 8 cc. of lead subacetate solution were used, most of the color was removed and the filtrate contained from 50 to 70 per cent of the globulin. It is important, therefore, to add no more lead

subacetate than is necessary to precipitate most of the color, since lead subacetate also precipitates the globulin.[6] About 3 cc. of lead subacetate solution per gram of globulin at a protein concentration of from 0.5 per cent to about 2 per cent has been found satisfactory. The opalescent filtrate from such preparations, which was yellow, was adjusted to about pH 4.5. Two per cent by weight of Hyflo Standard-cel was added, with stirring, and the mixture then filtered through a ¼-inch layer of Filter-cel on a Büchner funnel with suction. The clear yellow filtrate was found to contain no virus and but a small amount of inactive protein, all of the activity having been



Photograph by J. A. Carlile.

FIG. 1. Reproduction of a photomicrograph of crystalline globulin having the properties of tobacco-mosaic virus, which was isolated from infected Turkish tobacco plants. × 655.

[6] A brief description of an improved method for the purification of crude globulin, in which the use of lead subacetate is omitted, has been published. See Phytopath. 26: 108. 1936. (Abst).

adsorbed on the celite. The celite filter cake containing the active protein was then suspended in sufficient water to make a 1 or 2 per cent protein solution. The mixture, which was then adjusted to pH 8 and filtered with suction, gave an opalescent filtrate. The celite filter cake was again suspended in water at pH 8 and filtered in order to remove most of the active protein from the celite. The 2 extracts of the filter cake were combined. Usually 2 or 3 treatments with celite at pH 4.5 and elution at pH 8 were sufficient to yield practically colorless opalescent solutions of active protein.

Crystallization of the globulin in such solutions was accomplished by adding sufficient amounts of a saturated ammonium sulphate solution to cause a faint turbidity and then adding, very slowly, a solution of 5 per cent glacial acetic acid in 0.5 saturated ammonium sulphate, with continual stirring, until the hydrogen-ion concentration was pH 4.5. A solution of saturated ammonium sulphate was then added dropwise until a 20 per cent concentration of ammonium sulphate in the mixture was reached, when crystallization was completed. Figure 1 is a reproduction of a photomicrograph of the crystalline globulin. The crystals are needles about 0.02 mm. long. Smaller crystals may be obtained by causing the protein to come out of solution rapidly. It also was found possible to cause crystallization by adding a little saturated ammonium or magnesium sulphate to a solution of the globulin in 0.001 N sulphuric acid. Although vegetable globulins have been crystallized by dialysis of their solutions, such treatment failed to induce crystallization in the case of the active tobacco-mosaic virus globulin.

General Properties of the Crystalline Material

The crystalline material has the general properties of a protein. A solution containing 1 mg. per cc. gives a positive test with Millon's, biuret, xan-

TABLE 5.—*Elementary analysis and optical activity of crystalline tobacco-mosaic virus protein. Samples dialyzed against dilute* HCl, *precipitated with acetone, and dried over* P_2O_5 *in vacuo at 60° C.*

		C	H	N (Dumas)	N (Kjeldahl)	Cl	S	P	Ash
Per cent ash-free dry weight	1[a]	52.00	7.00	16.09					1.51
		52.38	6.94	16.17					1.77
	2[a]	53.14	6.90	16.36	16.55	0.50	0.00	0.00	1.49
		53.25	6.99	16.25	16.60	0.52	0.00	0.00	1.30
Optical activity, $[\alpha]_D^{20°}$ per mg. nitrogen. (Samples in dilute NaOH solution at pH 11.2 ± 0.1)							−0.44		
							−0.42		

[a] Sample No. 1 was dialyzed at pH 4.5 for 4 days, and sample No. 2 was dialyzed at pH 3.25 for 8 days.

thoproteic, glyoxylic acid, and Folin's tyrosine reagents. The material is precipitated from solution by 0.4 saturation with ammonium sulphate, saturation with magnesium sulphate, or by the addition of acetone, ethyl alcohol, or solutions of lead acetate, phosphotungstic acid, safranine, tannic acid, and trichloracetic acid. The protein is coagulated and the activity lost on heating to 94° C. The analysis and optical rotation of the material are given in table 5. The nitrogen content and optical rotation of the protein were unchanged after it had been subjected to 10 successive crystallizations.

The crystalline material is between 100 and 1000 times more active than ordinary juice preparations from diseased Turkish tobacco plants. The activity might be expected to lie in this range, since 0.2 per cent by weight of the extract from diseased plants was isolated in the form of crystals. Table 6 gives the results of several activity tests on the first crystalline protein that was obtained, on the same after 10 successive crystallizations, and on a sample of infectious juice pressed from fresh macerated plants. Most of the activity measurements were made by the half-leaf method, using plants of *Phaseolus vulgaris* L., *Nicotiana glutinosa,* and *N. langsdorffii* Schrank. Dilutions of a solution of the crystalline material were made with 0.1M phosphate at pH 7. The undiluted infectious juice preparation was compared with a 10^{-3} dilution or 1 mg. per cc. of the crystals. It may be seen that the crystalline material is slightly less than 1000 times as active as the infectious juice. It also may be seen that the crystalline protein was active at a dilution of 10^{-9} gm. per cc., and that in several tests it was impossible to detect a significant difference between the activity of protein crystallized once and the activity of protein crystallized 10 times. The disease produced in healthy Turkish tobacco plants on inoculation with suspensions of the lesions caused by a solution containing 10^{-9} or more grams of crystalline protein per cc. has always proved to be typical tobacco mosaic. Large amounts of virus were found in plants inoculated with a suspension from 1 lesion from a leaf of *P. vulgaris* previously inoculated with a solution containing 10^{-9} gm. per cc. of the crystals.

The crystalline material is practically insoluble in water, although readily soluble in dilute solutions of acids, alkalis, or salts. Most of the work on the crystalline material was done with solutions containing about 1 per cent protein by weight. Solutions containing from 0.1 per cent to 2 per cent protein have a decided opalescent appearance. Such solutions are fairly clear between pH 6 and 11, but below about pH 5 take on a dense whitish appearance which becomes very pronounced at about pH 3.2. If solutions of the protein are made more alkaline than about pH 11, the opalescence disappears and they become clear. However, such solutions were inactive and it was found by solubility tests that the protein had been denatured. The material is also denatured and the activity lost when solutions are made more

TABLE 6.—*Infectivity tests on crystalline globulin and on infectious juice*

	Test plant	No. of half-leaves	Method[a]	1–10[3][b]	1–10[4]	1–10[5]	1–10[6]	1–10[7]	1–10[8]	1–10[9]
1x crystallized	P.	12	L	139.5[c]	43.6	16.3	1.5	0.2	0.0	0.0
10x crystallized	vulgaris	12	R	178.1	32.0	25.8	1.6	0.4	0.1	0.0
1x crystallized	N.	10	L	67.1	65.0	14.2	4.4	0.9	0.1	0.0
10x crystallized	glutinosa	10	R	98.0	44.0	19.9	3.2	0.6	0.2	0.1
1x crystallized	N. langs-	5	L	179.0	160.0	86.0	9.4			
10x crystallized	dorffii	5	R	213.0	119.0	60.0	12.3			
10x crystallized	P.	12	L	95.5	49.9	2.5	2.3	0.6	0.2	0.0
1x crystallized	vulgaris	12	R	83.1	49.1	7.6	8.0	0.7	0.2	0.1
1x crystallized	P.	180	P			19.5				
10x crystallized	vulgaris	180	P			21.8				
Crystals	P.	24	W	131.9	99.3	13.4	2.5	0.7	0.2	0.1
	vulgaris	24	W	51.6	45.5	10.5	1.6	0.4	0.1	0.0
Crystals	P.	12	L	187.5	66.5	42.1	18.5	4.1	1.6	0.4
Infectious juice	vulgaris	12	R	168.5	85.0	41.5	17.6	8.2	4.7	1.4
Crystals	N.	10	L	22.4	26.2	22.7	2.5	1.0	0.1	
Infectious juice	glutinosa	10	R	21.0	31.8	11.7	1.5	0.7	0.1	

[a] L indicates inoculation was on left leaf-halves, R right leaf-halves of same leaves. P indicates one whole leaf of each plant was inoculated with 1x crystallized material and the other whole leaf of the same plant was inoculated with 10x crystallized material. W indicates that the whole leaf was inoculated.

[b] All dilutions were made with phosphate at pH 7. Designated dilutions of infectious juice should be divided by 1000. Undiluted juice was compared with a $1–10^3$ dilution of crystals and then both preparations were diluted equally. A $1–10^3$ dilution of crystals denotes a solution containing 0.001 gm. of protein per cc., $1–10^4$ denotes 0.0001 gm. of protein per cc., etc.

[c] Numbers represent the average number of lesions per half-leaf obtained on inoculation with the designated preparation and dilution.

acid than about pH 1. The active protein readily passes a Berkefeld "W" filter, but fails to pass collodion filters through which proteins, such as egg albumin, readily pass. It has not been found possible to separate the activity from the protein by means of collodion filters.

Table 7 records the results of a number of precipitin tests made with

TABLE 7.—*Results of precipitin tests with crystalline protein*

Dilution	Anti-crystalline protein guinea-pig serum	Normal guinea-pig serum	Anti-infectious tobacco juice rabbit serum	Normal rabbit serum	Anti-normal tobacco juice rabbit serum	Normal rabbit serum
Crystalline protein						
1–1,000	++++[a]	–	++++	–	–	–
1–10,000	++	–	++	–	–	–
1–50,000	±	–	+	–	–	–
1–100,000	–	–	±	–	–	–
1–200,000	–	–	–	–	–	–
Infectious juice from Turkish tobacco plants						
1	++	–	+++	–	–	–
1–5	+	–	+	±	–	–
1–10	–	–	+	–	–	–
1–50	–	–	±	–	–	–
1–100	–	–	–	–	–	–
Juice from normal Turkish tobacco plants						
1	–	–	–	–	+	–
1–5	–	–	–	–	±	–
1–10	–	–	–	–	±	–
1–50	–	–	–	–	–	–
1–100	–	–	–	–	–	–

[a] ++++ indicates a very heavy precipitate was obtained.
+++ indicates a heavy precipitate was obtained.
++ indicates a moderate precipitate was obtained.
+ indicates a slight precipitate was obtained.
± indicates a very slight precipitate was obtained.
– indicates no precipitate was obtained.

sera kindly prepared by Dr. K. S. Chester. It may be seen that the serum of an animal injected with a solution of the crystalline material gives a precipitate when mixed with infectious juice or with solutions of the crystals, and fails to give a reaction with the juice from normal tobacco plants. The sera of animals injected with infectious juice give a precipitate when mixed with a solution containing but 1 part in 100,000 of the crystalline protein, whereas the sera of animals injected with juice from normal tobacco plants

give no precipitate when mixed with a solution of the crystals. The fact that a solution of the crystals may be diluted so high and yet give a visible precipitate when mixed with immune serum indicates that the crystalline material is essentially pure and free from foreign material. The failure to obtain a precipitate when serum from animals injected with crystalline protein is mixed with an extract from normal plants, or when serum from animals injected with extracts from normal plants is mixed with a solution of crystalline material, is strong evidence that the active crystallizable protein does not exist in normal plants.

DISCUSSION

The isolation of a highly infectious crystalline protein from extracts of Turkish tobacco plants diseased with tobacco-mosaic virus brings to the forefront many problems having to do with viruses. Ever since 1898, when Beijerinck (2) introduced his theory of a contagious living fluid, much discussion has centered around the question as to whether or not viruses are alive. The evidence presented in this paper indicates that the virus of tobacco mosaic, the first to be described in the literature (7), is not alive. It is exceedingly difficult to conceive of a living agent that may be not only crystallized, but recrystallized repeatedly, with retention of constant physical, chemical, and biological properties. Such characteristics are regarded as those of pure chemical compounds. Hence it seems reasonable to assume that the crystalline protein is a chemical compound and, therefore, inanimate. The question then arises as to whether the virus activity is a property of the protein molecule, or is a property of something, living or nonliving, adsorbed on the protein. If a living agent is responsible for the activity, then, in view of the constant properties that the crystalline protein possesses, it certainly is not an ordinary contaminant. It may be possible that some hitherto unknown close association may exist between a crystalline protein and a living agent. On the basis of present knowledge, such an association seems very unlikely and it is mentioned only as a possibility. If a nonliving contaminant exists in the crystals, then it follows, of course, that the virus itself is nonliving. However, no evidence for the existence of a mixture of active and inactive material in the crystals has been obtained. Although it is difficult, if not impossible, to obtain conclusive positive proof of the purity of a protein, all of the evidence obtained up to the present time indicates that the crystalline material is pure. Since it is of great importance to know with as much certainty as possible whether or not the crystalline protein is pure, experiments from which it is hoped further evidence on this point will be obtained are now in progress.

The fact that this virus seems to be a protein may necessitate a fundamental change in the conception of the nature of other viruses. That a

chemical substance should possess properties that endow it with the disease-producing potentialities of tobacco-mosaic virus is most striking. Yet, when examined in the light of recent developments in protein chemistry, the phenomenon is not so surprising. A crystalline protein, trypsinogen, recently isolated by Kunitz and Northrop (10), is distinguished by its lack of hydrolytic properties. However, on addition of a little trypsin the inactive trypsinogen is converted into trypsin, an active hydrolytic agent. The activation reaction is autocatalytic and may be demonstrated *in vitro*. The tobacco-mosaic virus protein is somewhat different in that the presence of living cells appears to be necessary for multiplication of the protein. If the precursor of the virus protein should be an unstable or transient form of protein, existent only in association with the living cells of certain plants, then it will be possible to demonstrate catalysis or multiplication of virus only in the presence of such living cells. If, on the other hand, the precursor should be stable, then it should be possible to catalyze the production of virus protein *in vitro* by the addition of a little of the active protein to the precursor. Furthermore, it is conceivable that the precursor might exist in some plants and fail to exist in other plants. The latter group would be immune from the tobacco-mosaic disease, since virus protein would be expected to multiply only in hosts having the precursor of the active protein introduced. This may be the explanation of the host specificity of tobacco-mosaic virus.

On the basis that this virus is a protein, a chemical explanation of infection as the inauguration of an autocatalytic reaction and of the disease itself as a resultant of the disruption of normal protein metabolism caused by the production of an enormous amount of foreign protein appears feasible. The actual isolation of over 2 mg. of active protein per cubic centimeter of plant extract demonstrates that it is present in the plant material in relatively large amounts. The production in such large quantities brings up the question of how the virus protein spreads in plants. It seems likely that it moves rapidly in the phloem vessels (12). The cell-to-cell type of virus movement, which has been so difficult to account for in the past, might be readily explained if one assumes that active protein in one cell catalyzes the production of protein in adjacent cells without passing from cell to cell. Thus, although any one molecule might not move out of the cell in which it is produced, the result of such catalysis would be an apparent rapid movement due to contact of active protein at the periphery of one cell on inactive protein in adjacent cells. It is likely that diffusion and protoplasmic streaming play a part in the movement of active protein molecules, but it is difficult to account for the movement of such large molecules by diffusion and protoplasmic streaming alone. The nature of the intracellular inclusions found in plants infected with tobacco mosaic remains to be determined.

The bodies may consist of active protein, or of aggregates of essentially inactive protein separated during the production of virus protein.

The occurrence of many strains (8) of tobacco-mosaic virus has a fairly logical explanation on a chemical basis, since they may result from changes in the active molecule to give similar or isomeric proteins. It is well known that slightly unstable molecules may, under certain conditions, undergo rearrangement or polymerization to give molecules having different physical and chemical properties. In a molecule as large as the active protein, many different changes, giving rise to different molecules and hence to many different virus strains, might be expected. Such change in the structure of an active protein molecule might be regarded as an example of adaptation.

The isolation of a typical virus in the form of a crystalline protein permits many phases of the general virus problem to be viewed with a new significance. Although some workers hold that certain viruses are living agents, it appears reasonable to assume that a considerable number of them may be proteins similar in nature to the virus of tobacco mosaic. The multiplication, adaptation, and specificity of viruses, their possible dependence on the presence of living cells for multiplication, and even immunity, may be explained on a chemical basis without great difficulty.

SUMMARY

A crystalline protein, which has the properties of tobacco-mosaic virus, has been isolated from an extract of Turkish tobacco plants infected with this virus. The extract was prepared by grinding frozen plants, adding disodium phosphate, and pressing out the liquid. The press cake was extracted a second time with dilute disodium phosphate and the 2 extracts were filtered through celite and combined. The virus protein was obtained from these extracts by precipitation with ammonium sulphate. The virus protein was reprecipitated with ammonium sulphate several times with loss of much color, and most of the remaining color was then removed with lead subacetate. The virus was adsorbed on, and removed from, celite several times and then crystallized in the form of small needles about 0.02 mm. long by the addition of a solution of 5 per cent glacial acetic acid in 0.5 saturated ammonium sulphate.

The crystalline material has the general properties of a protein and its infectivity, chemical composition, and optical rotation were unchanged after 10 crystallizations. The material is between 100 and 1000 times more active than ordinary infectious juice preparations, one cubic centimeter of a solution containing 10^{-9} gm. per cc. of the protein usually proving infectious. The crystalline material reacts with sera of animals injected with a solution of the crystals or with infectious juice, and fails to react with the sera of animals injected with juice from normal plants. All of the evidence obtained

up to the present time indicates that the crystalline protein is pure or possibly a solid solution of proteins, and hence, that tobacco-mosaic virus is a protein.

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SEED-POTATO TREATMENT FOR RHIZOCTONIA CONTROL IN NORTHEASTERN MAINE, 1929 TO 1933[1]

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INTRODUCTION

The results of seed-potato treatment experiments in northeastern Maine for the years 1925 to 1928 were published by Schultz, Gratz, and Bonde (7). The experiments in the same locality for the years 1929 to 1933 were conducted in order to test the effectiveness of commercial products against rhizoctonia and, if possible, to develop new and more effective short-time treatments. The problem of soil infestation was given some consideration and information was obtained on the value of selection of clean seed potatoes for planting.

METHODS

In general, the methods used in these experiments were similar to those used by Schultz, Gratz, and Bonde (7). Three kinds of seed potatoes were used: Rhizoctonia-infected, clean, and field-run.

Rhizoctonia-infected tubers were used in order to get an index of the effectiveness of the treatments. They were selected at harvest time and only those with a high percentage of their surface covered by rhizoctonia sclerotia were taken. Most of the sclerotia were less than $\frac{1}{16}$ inch thick, which is typical of the rhizoctonia black-scurf stage of infection in northeastern Maine. All of the rhizoctonia-infected tubers used in these experiments were of the Irish Cobbler variety.

The clean tubers were used to give an index of possible injurious effects of the treatments and to give an index of soil infestation by rhizoctonia. Only tubers that showed no rhizoctonia sclerotia when washed or brushed were used as clean seed. It is probable that some of these tubers were lightly infected by rhizoctonia and escaped notice. In all experiments on rhizoctonia-infected seed a nontreated clean check and a treated clean check, as used by Melhus and Gilman (4), were included. From 1930 to 1933 separate experiments were conducted in which only clean tubers were used. This method largely avoids the possibility of field spread from the diseased rows to the clean ones and therefore gives a good index of soil infestation. Clean, $1\frac{1}{2}$-ounce whole tubers were included in the clean Irish

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Cobbler experiments in 1931, 1932, and 1933 as a possible check on seed-piece decay.

The field-run tubers were used in order to secure data on the value of treatment of what might be considered average commercial Green Mountains in northeastern Maine. They were graded and only primes were used for seed, but no selection regarding rhizoctonia was made. They were grown from treated tubers and usually bore rhizoctonia sclerotia on less than 10 per cent of the tubers. In general, the Green Mountain potatoes grown in Maine are not so heavily infected with rhizoctonia as are the Irish Cobblers.

The seed tubers, with the exception of a few cases where they were treated in the fall, were planted shortly after being treated. This interval varied from 5 to 19 days and was usually less than 2 weeks. All treatments were made before the tubers were cut.

The treatments varied in length from dip treatments to 2-hour soaks. By a dip treatment is meant that the tubers either were dipped 3 times in rapid succession into a solution or were placed in a solution, agitated slightly, and then removed from the solution. By either of these methods the tubers are more thoroughly wetted than if they are merely dipped once into a solution, especially if they are covered with a film of dust. After treatment the tubers were placed in bushel crates that allowed the passage of air and aided in drying them.

Throughout the 5-year period, all tubers, except those planted whole, were cut by one man to as nearly 1½-ounce seed pieces as possible.

Only seed tubers from a common source were included in each experiment.

In all cases 10 plots of 28 hills each were planted for yield. Just before harvest the end hills of these plots were removed, leaving 26 hills. The plots for yield were arranged according to the chessboard plan, a modification of the magic-square system, as employed by Schultz, Gratz, and Bonde (7) in 1927 and 1928. Two extra plots for examination for stem lesions and seed-piece decay were planted outside the buffer rows of each experiment.

All plots were planted on Aroostock Farm, Presque Isle, Maine, on land that was in a 3-year rotation of oats, clover, and potatoes. The most uniform land available was selected and commercial fertilizer was applied, at the rate of about 2000 pounds per acre, in the rows with a planter that mixed it with the soil. The date of planting varied from May 11 to May 23.

All seed pieces were dropped by hand at 14-inch intervals in rows approximately 3 feet apart. The seed pieces were covered with a horse-hoe. In the years 1929, 1930, and 1931 they were covered to a depth of about 6 inches, while in 1932 and 1933 they were covered to a depth of about 4½ inches.

The plants in the extra rows usually were about 4 inches tall when they

were dug and examined for seed-piece decay and stem lesions. Since only a very few decayed seed pieces were found in any of the experiments, the data on such decay are omitted from the tables.

Notes were taken on stand and vigor usually when the plants were from 6 to 10 inches tall. Regarding vigor, the height of each plant was estimated and the relative vigor, as recorded in the tables, was determined from these estimates.

All plots were cultivated and sprayed as uniformly as possible. Considerable variation in injury to the different plots was encountered in 1930 with the use of a tractor in spraying.

The plots were harvested soon after the plants had died. The tubers were graded according to the standards of the United States Department of Agriculture and figures were obtained on primes, seconds, and culls. For yield comparisons, only primes and seconds were used and these are referred to as marketable tubers. Except in the experiments where clean tubers were planted and the potatoes harvested had only a very low percentage of infection, 100 tubers from each plot were taken at random, washed, and examined for rhizoctonia sclerotia. This made 1,000 tubers for each treatment. All tubers showing any rhizoctonia sclerotia were classed as infected. According to the work of Bisby, Higham, and Groh (1), it is probable that rhizoctonia infection might have been heavier had the plots been harvested later.

The formulae $\text{PEM} = \frac{.675 \times \text{standard deviation}}{\sqrt{\text{No. of items}}}$

and $\text{PED} = \sqrt{a^2 + b^2}$, where

a = probable error of the mean of the treated lot and b = probable error of the check with which the treated lot is compared, were used for determining the significance of the differences in yield. Odds as listed by Pearl and Miner (5) were used.

1929 EXPERIMENTS

The results of experiments conducted in 1929 are given in tables 1 and 2. Nine treatments were compared with an untreated check on rhizoctonia-infected and clean Irish Cobblers of a common origin and included in one experiment. The same 9 treatments were used on field-run Green Mountains in comparison with a nontreated check and with a treated and a nontreated lot of clean Green Mountains from the same source. The treatments used are listed in tables 1 and 2. The last 3 materials listed in table 1 were experimental products furnished by the Bayer-Semesan Company.

Of the treatments used, K 8 was the most injurious, as indicated by the relative vigor of the plants from seed treated with this material. Clean, nontreated Irish Cobblers showed a relative vigor of 96, while those treated

TABLE 1.—*Seed-potato treatment results—Irish Cobblers 1929*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over diseased nontreated check	
							Bushels	Approximate odds
Diseased, check, not treated		99.6	84	71	79	261.7 ± 5.5		
Diseased, corrosive sublimate 1–1,000	1½ hrs.	99.2	95	2	13	309.5 ± 3.9	47.8 ± 6.7	434,782: 1
Diseased, corrosive sublimate 1–500	3 min.	99.6	95	10	20	294.0 ± 3.7	32.3 ± 6.6	1,052: 1
Diseased, corrosive sublimate 1–500 and 0.8% glacial acetic acid	3 min.	99.2	96	4	8	305.2 ± 4.8	43.5 ± 7.3	19,230: 1
Diseased, formaldehyde 1–120, 52° C.	3 min.	98.5	92	16	28	289.6 ± 6.6	27.9 ± 8.6	31: 1
Diseased, formaldehyde 1–120, 11° C.	3 min.	98.8	94	12	14	309.1 ± 6.5	47.4 ± 8.5	19,230: 1
Diseased, Semesan Bel 1–20	1 min.	99.2	93	29	52	298.0 ± 4.5	36.3 ± 7.1	1,350: 1
Diseased, Cal K 1–40	1 min.	99.6	92	2	5	301.6 ± 6.0	39.9 ± 8.1	1,052: 1
Diseased, K 8 1–40	1 min.	96.5	77	0	3	269.3 ± 4.5	7.6 ± 7.1	1: 1
Diseased, Dubay No. 664 1–68	1 min.	99.6	96	4	9	305.6 ± 5.2	43.9 ± 7.6	19,230: 1
Clean, check, not treated		100	96	2	2	312.3 ± 5.6	50.6 ± 7.8	19,230: 1
Clean, corrosive sublimate 1–1000	1½ hrs.	99.6	95	2	1	301.6 ± 6.1	39.9 ± 8.2	1,052: 1
Clean, corrosive sublimate 1–500	3 min.	98.8	96	0	3	310.3 ± 4.0	48.6 ± 6.8	434,782: 1
Clean, corrosive sublimate 1–500 and 0.8% glacial acetic acid	3 min.	100	97	0	3	308.7 ± 5.5	47.0 ± 7.8	19,230: 1
Clean, formaldehyde 1–120, 52° C.	3 min.	98.8	98	0	2	313.5 ± 5.5	51.8 ± 7.8	434,782: 1
Clean, formaldehyde 1–120, 11° C.	3 min.	99.6	96	2	1	298.4 ± 3.3	36.7 ± 6.4	19,230: 1
Clean, Semesan Bel 1–20	1 min.	100	96	0	2	316.7 ± 5.8	55.0 ± 8.0	434,782: 1
Clean Cal K 1–40	1 min.	100	92	2	3	293.6 ± 5.4	31.9 ± 7.7	175: 1
Clean K 8 1–40	1 min.	97.7	78	0	2	266.5 ± 5.2	4.8 ± 7.6	0
Clean Dubay No. 664 1–68	1 min.	99.2	96	0	1	309.5 ± 6.1	47.8 ± 8.2	19,230: 1

TABLE 2.—*Seed-potato treatment results—Green Mountains 1929*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over field-run nontreated check	
							Bushels	Approximate odds
Field-run, check, not treated		99.2	99	23	6	318.7 ± 5.6	.....	.....
Field-run, corrosive sublimate 1–1000	1½ hrs.	99.2	99	10	1	327.5 ± 5.5	8.8 ± 7.8	1 : 1
Field-run, corrosive sublimate 1–500	3 min.	99.6	99	12	4	321.1 ± 6.3	2.4 ± 8.4	0
Field-run, corrosive sublimate 1–500 and .8% glacial acetic acid	3 min.	96.9	98	2	1	316.3 ± 8.1	−2.4 ± 9.8	0
Field-run, formaldehyde 1–120, 52° C.	3 min.	96.9	97	8	6	301.9 ± 5.1	−16.8 ± 7.6	6 : 1
Field-run, formaldehyde 1–120, 11° C.	3 min.	100	98	6	2	320.3 ± 6.0	1.6 ± 8.2	0
Field-run, Semesan Bel 1–20	1 min.	98.8	98	17	8	321.1 ± 5.5	2.4 ± 7.8	0
Field-run, Cal K 1–40	1 min.	99.2	98	2	1	314.7 ± 6.6	−4.0 ± 8.7	0
Field-run, K 8 1–40	1 min.	98.8	95	5	1	300.8 ± 5.6	−17.9 ± 7.9	7 : 1
Field-run, Dubay No. 664 1–68	1 min.	99.2	98	4	2	315.1 ± 7.4	−3.6 ± 9.3	0
Clean, check, not treated		100	99	6	3	319.5 ± 8.5	0.8 ± 10.2	0
Clean, corrosive sublimate 1–1000	1½ hrs.	99.2	100	8	1	321.5 ± 5.2	2.8 ± 7.6	0

TABLE 3.—*Seed-potato treatment results—Irish Cobblers 1930*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over diseased nontreated check	
							Bushels	Approximate odds
Diseased, check, not treated		98.8	85	88	82	402.5 ± 8.8		
Diseased, corrosive subimate 1–1000	1½ hrs.	100	94	15	11	439.6 ± 12.8	37.1 ± 15.5	8: 1
Diseased, corrosive sublimate 1–500	3 min.	99.2	88	23	52	432.0 ± 6.3	29.5 ± 10.8	14: 1
Diseased, corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	100	92	10	16	442.4 ± 6.2	39.9 ± 10.8	78: 1
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	100	93	12	10	450.4 ± 8.0	47.9 ± 11.9	142: 1
Diseased, formaldehyde 1–120, 52° C. covered 1 hr.	3 min.	100	94	10	34	452.4 ± 9.1	49.9 ± 12.7	116: 1
Diseased, formaldehyde 1–120, 10° C. covered 1 hr.	3 min.	99.6	88	56	70	423.6 ± 9.3	21.1 ± 12.8	3: 1
Diseased, formaldehyde 1–240	2 hrs.	99.6	87	48	66	414.5 ± 6.0	12.0 ± 10.7	1: 1
Diseased, Dubay No. 664 1–64	dip	99.6	93	35	35	440.0 ± 11.3	37.5 ± 14.3	12: 1
Diseased, Dubay No. 693 1–64	dip	99.6	92	6	17	448.8 ± 10.7	46.3 ± 13.9	37: 1
Diseased, Dubay No. 738 1–80	dip	97.3	82	2	8	433.6 ± 8.0	31.1 ± 11.9	12: 1
Diseased, Sanosced 1–60	dip	99.6	95	10	15	441.6 ± 11.5	39.1 ± 14.5	14: 1
Clean, check, not treated		99.2	95	6	2	429.2 ± 11.4	26.7 ± 14.4	4: 1
Clean, corrosive sublimate 1–1000	1½ hrs.	99.2	93	2	4	435.2 ± 9.7	32.7 ± 13.1	10: 1

TABLE 4.—*Seed-potato treatment results—clean Irish Cobblers 1930*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over nontreated check	
							Bushels	Approximate odds
Check, not treated		100	96	2	1	438.8 ± 9.4	.....	.....
Corrosive sublimate 1–1000	1½ hrs.	99.6	93	0		453.9 ± 6.6	15.1 ± 11.5	2:1
Corrosive sublimate 1–500	3 min.	99.6	94	0		457.9 ± 8.8	19.1 ± 12.9	2:1
Corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	100	95	0		461.9 ± 7.5	23.1 ± 12.0	4:1
Corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	100	95	0		448.0 ± 7.8	9.2 ± 12.2	0
Formaldehyde 1–120, 5[illegible] C.	3 min., covered 1 hr.	99.6	95	0		441.2 ± 6.6	2.4 ± 11.5	0
Formaldehyde 1–120, 10° C.	3 min., covered 1 hr.	100	95	0		435.2 ± 6.5	−3.6 ± 11.4	0
Formaldehyde 1–240	2 hrs.	100	96	0		437.6 ± 12.8	−1.2 ± 15.9	0
Dubay No. 664 1–64	dip	100	95	0		463.5 ± 8.2	24.7 ± 12.5	5:1
Dubay No. 693 1–64	dip	100	94	0		453.2 ± 7.8	14.4 ± 12.2	1:1
Dubay No. 738 1–80	dip	98.8	85	0		421.6 ± 7.7	−17.2 ± 12.2	2:1
Sanoseed 1–60	dip	100	93	0		439.6 ± 6.9	0.8 ± 11.7	0

TABLE 5.—*Seed-potato treatment results—Green Mountains 1930*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over field-run nontreated check	
							Bushels	Approximate odds
Field-run, check, not treated		99.6	98	21	13	434.4 ± 9.8	.....	.....
Field-run, corrosive sublimate 1–1000	1½ hrs.	99.2	99	10		463.5 ± 11.6	29.1 ± 15.2	4: 1
Field-run, corrosive sublimate 1–500	3 min.	100	98	8		443.6 ± 8.3	9.2 ± 12.8	0
Field-run, corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	100	99	2		446.4 ± 5.7	12.0 ± 11.3	1: 1
Field-run, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	99.6	97	0		430.8 ± 8.5	−3.6 ± 13.0	0
Field-run, formaldehyde 1–120, 52° C.	3 min., covered 1 hr.	100	99	2		461.9 ± 8.9	27.5 ± 13.2	5: 1
Field-run, formaldehyde 1–120, 10° C.	3 min., covered 1 hr.	98.5	98	6		432.0 ± 7.8	−2.4 ± 12.5	0
Field-run, formaldehyde 1–240	2 hrs.	99.2	98	4		415.7 ± 8.9	−18.7 ± 13.2	2: 1
Field-run, Dubay No. 664 1–64	dip	99.6	98	25		429.6 ± 9.9	−4.8 ± 13.9	0
Field-run, Dubay No. 693 1–64	dip	98.5	98	4		434.0 ± 8.8	−0.4 ± 13.2	0
Field-run, Dubay No. 738 1–80	dip	100	95	0		448.4 ± 8.6	14.0 ± 13.0	1: 1
Field-run, Sanoseed 1–60	dip	100	98	0		445.2 ± 7.4	10.8 ± 12.3	0
Clean, check, not treated		100	99	4	7	454.7 ± 9.8	20.3 ± 13.9	2: 1
Clean, corrosive sublimate 1–1000	1½ hrs.	99.2	98	0		426.4 ± 9.9	−8.0 ± 13.9	0

with K 8 showed a relative vigor of 78. Also, the yield of the clean Irish Cobblers was much lower with K 8 than with any other treatment.

With the exception of K 8, all of the treatments on the rhizoctonia-infected seed gave significant increases in yield ranging from 27.9 to 47.8 bushels per acre.

Semesan Bel was decidedly inferior in its control of Rhizoctonia. For hot formaldehyde also there was 28 per cent tuber infection compared with 13 per cent for the standard corrosive sublimate. In these tests the usual 1-hour covering following the hot formaldehyde treatment was omitted; therefore, these results cannot be accepted as representative of the regular hot-formaldehyde treatment.

Only 2 of the treated lots of clean Irish Cobblers yielded more than the nontreated check, and these differences were not significant.

On the field-run Green Mountains most of the treatments decreased the number of stem lesions and the amount of tuber infection, but there was no significant difference between the yield from the nontreated check and that from any of the treated lots.

1930 EXPERIMENTS

The 1930 experiments were similar to those conducted in 1929 except that clean Irish Cobblers were planted in a separate experiment. The results are recorded in tables 3, 4, and 5. The 3 numbered materials listed in the tables under the name Dubay were experimental products furnished by the Bayer-Semesan Company.

The acid corrosive sublimate treatment (corrosive sublimate 1–500 with 1 per cent commercial hydrochloric acid) was the same as that used by Leach, Johnson, and Parson (3), except that the time of treatment was 3 minutes instead of 5.

The strength of the glacial acetic acid was changed from the 0.8 per cent used in 1929 to 0.56 per cent, since that strength gave about the same acidity as determined by titrating against an alkali as 1 per cent commercial hydrochloric acid as used in the regular acidulated corrosive sublimate treatment.

The experimental product Dubay No. 664 did not control rhizoctonia so well as in 1929. According to information received from the company, the 1930 product was made by a different process than the 1929 product and apparently was not identical with it.

All treatments used on Rhizoctonia-infected tubers gave increased yields, but the increases were statistically significant in only 4 instances.

None of the treatments caused a significant difference in yield of either the clean Irish Cobblers or the field-run Green Mountains.

In 1930 the plots were sprayed with a tractor, which caused marked injury in some instances and accounted for part of the variability recorded.

TABLE 6.—*Seed-potato treatment results—Irish Cobblers 1931*

Treatment	Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over diseased nontreated check	
						Bushels	Approximate odds
Diseased, check, not treated	99.6	84	71	60	321.1 ± 11.5	.....	.....
Diseased, corrosive sublimate 1–1000 1½ hrs.	99.2	95	8	26	338.7 ± 10.8	17.6 ± 15.8	1:1
Diseased, corrosive sublimate 1–500 3 min.	100	94	40	43	351.4 ± 11.0	30.3 ± 15.9	4:1
Diseased, corrosive sublimate 1–500 and .56% glacial acetic acid 3 min.	99.2	93	37	31	333.9 ± 9.8	12.8 ± 15.1	0
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid 3 min.	97.7	96	10	25	354.6 ± 11.9	33.5 ± 16.5	5:1
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid dip	98.5	94	29	36	331.1 ± 11.2	10.0 ± 16.1	0
Diseased, formaldehyde 1–120, 52° C. 3 min., covered 1 hr.	97.3	85	38	34	345.4 ± 9.9	24.3 ± 15.2	3:1
Diseased, formaldehyde 1–120, 8° C. 3 min., covered 1 hr.	98.8	85	63	57	314.3 ± 10.1	−6.8 ± 15.3	0
Diseased, formaldehyde 1–240 2 hrs.	100	92	48	35	365.4 ± 6.1	44.3 ± 13.0	45:1
Diseased, New Improved Semesan Bel 1–60 dip	99.6	95	35	36	341.5 ± 13.2	20.4 ± 17.5	1:1
Diseased, Sanoseed 1–60 dip	99.6	97	23	27	356.2 ± 6.1	35.1 ± 13.0	14:1
Diseased, yellow mercuric oxide 1–100 dip	100	88	0	11	322.3 ± 9.7	1.2 ± 15.0	0
Diseased, corrosive sublimate 1–1200 and potassium iodide 1–400 dip	100	96	10	31	367.0 ± 8.3	45.9 ± 14.2	31:1
Diseased, corrosive sublimate 1–500 and ammonium carbonate 1–40 3 min.	98.8	96	21	29	338.3 ± 10.0	17.2 ± 15.3	1:1
Diseased, corrosive sublimate 1–1000 treated 9/22/30 1½ hrs.	100	96	15	28	364.2 ± 9.8	43.1 ± 15.1	19:1
Clean, check, not treated	100	97	8	16	360.6 ± 10.3	39.5 ± 15.4	12:1
Clean, corrosive sublimate 1–1000 1½ hrs.	100	96	4	13	350.6 ± 10.7	29.5 ± 15.7	4:1

TABLE 7.—*Seed-potato treatment results—clean Irish Cobblers 1931*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over non-treated check	
							Bushels	Approximate odds
Check, not treated		99.2	99	0	1	399.7 ± 8.1	.....	.....
Corrosive sublimate 1–1000	1½ hrs.	98.1	98	4	1	401.3 ± 8.5	1.6 ± 11.7	0
Corrosive sublimate 1–500	3 min.	100	98	2		402.9 ± 8.5	3.2 ± 11.7	0
Corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	97.3	97	2		406.1 ± 7.3	6.4 ± 10.9	0
Corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	98.8	98	2		411.7 ± 10.8	12.0 ± 13.5	0
Corrosive sublimate 1–500 and 1% commercial hydrochloric acid	dip	98.1	98	2		411.3 ± 6.8	11.6 ± 10.6	1: 1
Formaldehyde 1–120, 52° C. 3 min., covered 1 hr.		93.1	94	2		386.9 ± 9.8	–12.8 ± 12.7	1: 1
Formaldehyde 1–120, 8° C. 3 min., covered 1 hr.		95.8	98	0		397.7 ± 6.4	–2.0 ± 10.3	0
Formaldehyde 1–240	2 hrs.	97.3	98	2		404.9 ± 9.9	5.2 ± 12.8	0
New Improved Semesan Bel 1–60	dip	99.6	98	0		398.1 ± 8.0	–1.6 ± 11.4	0
Sanoseed 1–60	dip	98.1	99	2		396.9 ± 7.1	–2.8 ± 10.8	0
Yellow mercuric oxide 1–100	dip	98.8	95	0		388.9 ± 8.4	–10.8 ± 11.7	0
Corrosive sublimate 1–1200 and potassium iodide 1–400	dip	99.2	98	6		402.1 ± 7.8	2.4 ± 11.2	0
Corrosive sublimate 1–500 and ammonium carbonate 1–40	3 min.	99.2	98	0		394.5 ± 7.5	–5.2 ± 11.0	0
1½-oz. whole tubers, check, not treated		98.8	100	2		435.2 ± 9.3	35.5 ± 12.3	19: 1
1½-oz. whole tubers, corrosive sublimate 1–1000	1½ hrs.	99.6	99	4		413.3 ± 7.1	13.6 ± 10.8	2: 1

TABLE 8.—*Seed-potato treatment results—Green Mountains 1931*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over field-run nontreated check	
							Bushels	Approximate odds
Field-run, check, not treated		100	99	4	2	431.6 ± 8.6	.....	.....
Field-run, corrosive sublimate 1–1000	1½ hrs.	100	99	0	3	426.8 ± 9.3	−4.8 ± 12.7	0
Field-run, corrosive sublimate 1–500	3 min.	99.6	99	0		416.5 ± 6.9	−15.1 ± 11.0	2: 1
Field-run, corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	98.8	99	0		409.3 ± 7.9	−22.3 ± 11.7	4: 1
Field-run, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	97.3	97	0		417.6 ± 7.0	−14.0 ± 11.1	2: 1
Field-run, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	dip	99.6	98	0		434.4 ± 8.7	2.8 ± 12.2	0
Field-run, formaldehyde 1–120, 52° C.	3 min., covered 1 hr.	93.5	91	0		388.5 ± 6.6	−43.1 ± 10.8	142: 1
Field-run, formaldehyde 1–120, 8° C.	3 min., covered 1 hr.	98.8	98	8		425.6 ± 9.8	−6.0 ± 13.0	0
Field-run, formaldehyde 1–240	2 hrs.	99.6	99	0		440.0 ± 10.2	8.4 ± 13.3	0
Field-run, New Improved Semesan Bel 1–60	dip	99.6	99	0		434.4 ± 9.5	2.8 ± 12.8	0
Field-run, Sanoseed 1–60	dip	100	100	0		439.6 ± 8.0	8.0 ± 11.7	0
Field-run, yellow mercuric oxide 1–100	dip	100	98	0		430.4 ± 9.0	−1.2 ± 12.4	0
Field-run, corrosive sublimate 1–1200 and potassium iodide 1–400	dip	100	99	0		429.2 ± 10.6	−2.4 ± 13.6	0
Field-run, corrosive sublimate 1–500 and ammonium carbonate 1–40	3 min.	99.2	99	0		431.2 ± 9.2	−0.4 ± 12.6	0
Field-run, corrosive sublimate 1–1000 treated 9/22/30	1½ hrs.	100	99	0		431.2 ± 8.9	−0.4 ± 12.4	0
Clean, check, not treated		100	100	0	4	428.4 ± 7.2	−3.2 ± 11.2	0
Clean, corrosive sublimate 1–1000	1½ hrs.	98.8	99	4	0	434.4 ± 5.9	2.8 ± 10.4	0

1931 EXPERIMENTS

The results of the experiments conducted in 1931 are recorded in tables 6, 7, and 8. The seed tubers were given the corrosive sublimate and formaldehyde treatments, as in 1930; New Improved Semesan Bel, which was the same as Dubay No. 664 used in 1929; Sanoseed; yellow mercuric oxide 1–100 as a dip as used by Blodgett (2); a solution of corrosive sublimate 1–1200 and potassium iodide 1–400 used as a dip; and corrosive sublimate 1–500 and ammonium carbonate 1–40 used for 3 minutes. The acid corrosive sublimate was used both as a dip and as a 3-minute treatment. The standard corrosive sublimate was used as a fall treatment as well as a spring treatment. The land on which the rhizoctonia-infected Irish Cobblers were they were placed in storage in the fall rather than following removal from storage in the spring, as is the usual practice. White (9) reported good results with fall treatment in Kansas.

Most of the tubers treated in 1931 had sprouts ranging from $\frac{1}{8}$ inch to $\frac{3}{8}$ inch in length. This sprouted condition of the tubers probably was responsible for the injury that resulted in 1931 from some of the treatments. Most of the treatments caused some burning of the sprouts, and the hot formaldehyde seemed to be especially severe in this respect.

In the experiments of 1931 the seed was planted from 5 to 7 days after treatment. The land on which the rhizoctonia-infected Irish Cobblers were planted appeared uniform, but the variation in the yields from the different plots indicated that it was very uneven with respect to productivity.

The control of rhizoctonia, as measured by the infection on the tubers that were produced, was not so good as that obtained in other years. Clean seed tubers included in the experiment on rhizoctonia-infected tubers (Table 6) produced a crop with 16 per cent of the tubers infected, while clean seed tubers in an adjoining experiment (Table 7) in which only clean tubers were used produced a crop with only 1 per cent of the tubers infected. These data suggest that there may have been some spread of rhizoctonia from the diseased to the healthy plots in the experiment recorded in table 6. All of the treatments except the short-time one in cold formaldehyde gave yield increases from the rhizoctonia-infected seed tubers, but the increases apparently were significant only where the regular cold formaldehyde and the corrosive sublimate-potassium iodide treatments were used.

Seed treatment of the clean Irish Cobblers or of the field-run Green Mountains did not result in any significant increases in yield.

In the experiment on clean Irish Cobblers a treated and a nontreated lot of clean approximately 1½-ounce whole tubers were used as a possible check against seed-piece decay. The amount of seed-piece decay recorded in the experiments of 1931 was negligible, so no advantage for the whole tubers was noted in this regard. The yield of the nontreated whole tubers, how-

TABLE 9.—*Seed-potato treatment results—Irish Cobblers 1932*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over diseased nontreated check	
							Bushels	Approximate odds
Diseased, check, not treated		98.8	89	35	37	335.5 ± 9.2	.....	.....
Diseased, corrosive sublimate 1–1000	1½ hrs.	99.6	92	0	4	355.4 ± 3.2	19.9 ± 9.7	5: 1
Diseased, corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	100	95	8	16	375.4 ± 5.2	39.9 ± 10.6	95: 1
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	100	93	8	8	355.4 ± 5.1	19.9 ± 10.5	4: 1
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	dip	100	94	4	14	352.2 ± 6.2	16.7 ± 11.1	2: 1
Diseased, formaldehyde 1–120, 52° C.	3 min., covered 1 hr.	100	94	10	11	352.2 ± 8.4	16.7 ± 12.5	2: 1
Diseased, New Improved Semesan Bel 1–60	dip	99.2	97	10	16	353.0 ± 5.7	17.5 ± 10.8	3: 1
Diseased, Sanoseed 1–60	dip	100	95	8	12	358.6 ± 5.3	23.1 ± 10.6	6: 1
Diseased, yellow mercuric oxide 1–100	dip	98.8	78	0	3	310.3 ± 8.0	−25.2 ± 12.2	5: 1
Diseased, corrosive sublimate 1–1200 and potassium iodide 1–400	dip	100	95	6	6	348.6 ± 4.2	13.1 ± 10.1	2: 1
Diseased, corrosive sublimate 1–500 and ammonium carbonate 1–20	3 min.	100	93	8	16	334.7 ± 8.4	−0.8 ± 12.5	0
Diseased, corrosive sublimate 1–1000 treated 9/22/31	1½ hrs.	99.6	93	4	8	349.4 ± 3.9	13.9 ± 10.0	0
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid treated 9/22/31	3 min.	99.6	87	2	5	351.0 ± 5.2	15.5 ± 10.6	2: 1
Clean, check, not treated		99.6	98	0	3	367.4 ± 4.1	31.9 ± 10.1	31: 1
Clean, corrosive sublimate 1–1000	1½ hrs.	100	95	2	4	363.0 ± 6.1	27.5 ± 11.0	10: 1

TABLE 10.—*Seed-potato treatment results—clean Irish Cobblers 1932*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over non-treated check	
							Bushels	Approximate odds
Check, not treated		99.6	97	0	2	328.7 ± 5.9	.....	.....
Corrosive sublimate 1–1000	1½ hrs.	99.6	96	0	7	308.3 ± 11.6	−20.4 ± 13.0	3 : 1
Corrosive sublimate 1–500 and .56% glacial acetic acid	3 min.	100	97	4		321.1 ± 6.0	−7.6 ± 8.4	0
Corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	100	94	0		302.8 ± 5.4	−25.9 ± 8.0	31 : 1
Corrosive sublimate 1–500 and 1% commercial hydrochloric acid	dip	98.8	98	0		325.5 ± 3.1	−3.2 ± 6.7	0
Formaldehyde 1–120, 52° C. 3 min., covered	1 hr.	99.6	97	0		323.9 ± 5.3	−4.8 ± 7.9	0
New Improved Semesan Bel 1–60	dip	99.6	96	0		322.3 ± 5.7	−6.4 ± 8.2	0
Sanoseed 1–60	dip	100	96	4		325.5 ± 4.5	−3.2 ± 7.4	0
Yellow mercuric oxide 1–100	dip	99.2	88	0		302.8 ± 5.7	−25.9 ± 8.2	31 : 1
Corrosive sublimate 1–1200 and potassium iodide 1–400	dip	99.6	97	0		312.7 ± 3.2	−16.0 ± 6.7	8 : 1
Corrosive sublimate 1–500 and ammonium carbonate 1–20	3 min.	100	96	0		321.5 ± 6.0	−7.2 ± 8.4	0
1½-oz. whole tubers, check, not treated		100	99	0		343.5 ± 5.4	14.8 ± 8.0	4 : 1
1½-oz. whole tubers, corrosive sublimate 1–1000	1½ hrs.	100	98	0		329.1 ± 7.3	0.4 ± 9.4	0

TABLE 11.—*Seed-potato treatment results—Green Mountains 1932*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over field-run nontreated check	
							Bushels	Approximate odds
Feld-run, check, not treated		100	99	19	6	386.5 ± 7.9	.....	.....
Field-run, corrosive sublimate 1–1000	1½ hrs.	99.6	98	6	2	380.2 ± 7.1	−6.3 ± 10.6	0
Field-run, corrosive sublimate 1–500 and .56% glacial acetic acid	2 min.	100	100	0		387.3 ± 5.9	0.8 ± 9.9	0
Field-run, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	100	100	0		379.8 ± 4.9	−6.7 ± 8.3	0
Field-run, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	dip	100	100	2		375.4 ± 4.6	−11.1 ± 9.1	1:1
Field-run, formaldehyde 1–120, 52° C.	3 min., covered 1 hr.	99.6	100	4		397.3 ± 6.6	10.8 ± 10.3	1:1
Field-run, New Improved Semesan Bel 1–60	dip	99.6	100	8		379.8 ± 3.1	−6.7 ± 8.5	0
Field-run, Sanoseed 1–60	dip	100	100	0		392.9 ± 6.4	6.4 ± 10.2	0
Field-run, yellow mercuric oxide 1–100	dip	100	99	2		382.5 ± 4.5	−4.0 ± 9.1	0
Field-run, corrosive sublimate 1–1200 and potassium iodide 1–400	dip	100	100	0		407.3 ± 4.4	20.8 ± 9.0	7:1
Field-run, corrosive sublimate 1–500 and ammonium carbonate 1–20	3 min.	100	100	0		401.7 ± 5.9	15.2 ± 9.9	2:1
Field-run, corrosive sublimate 1–1000 treated 9/22/31	1½ hrs.	100	100	0		406.1 ± 5.9	19.6 ± 9.9	5:1
Field-run, corrosive sublimate 1–500 and 1% commercial hydrochloric acid treated 9/22/31	3 min.	100	99	2		390.1 ± 6.2	3.6 ± 10.0	0
Clean, check, not treated		99.6	100	4	1	401.3 ± 4.4	14.8 ± 9.0	3:1
Clean, corrosive sublimate 1–1000	1½ hrs.	99.6	100	12	1	399.3 ± 7.4	12.8 ± 10.8	1:1

ever, was 35.5 bushels per acre greater than that from the nontreated cut tubers, but the odds were only approximately 19 to 1.

1932 EXPERIMENTS

The experiments in 1932 were much the same as those of 1931. Tables 9, 10, and 11 give the results. In the experiment where infected tubers were used the injury due to rhizoctonia was not so great as that occurring in previous years. In the nontreated check only 35 per cent of the plants showed stem lesions and only 37 per cent of the tubers that were produced showed rhizoctonia sclerotia. Most of the treatments used reduced these percentages to relatively low figures. The seed pieces were covered to a depth of about 4½ inches as compared to about 6 inches in previous years.

The corrosive sublimate with the glacial acetic acid was the only treatment that caused a significant increase in yield of the rhizoctonia-infected Irish Cobblers but its control of rhizoctonia was not good, as indicated by the fact that 16 per cent of the tubers harvested bore rhizoctonia sclerotia.

All of the treated lots of clean Irish Cobblers yielded less than the nontreated check but the differences apparently were significant only where the acid corrosive sublimate and the yellow mercuric oxide treatments were used.

The treaments did not cause any significant difference in the yield of the field-run Green Mountains.

1933 EXPERIMENTS

The results of experiments of 1933 are given in tables 12 and 13. The treatments used in 1933 on rhizoctonia-infected Irish Cobblers included the standard corrosive sublimate, the acid corrosive sublimate and various modifications of it, and 4 treatments using corrosive sublimate and potassium iodide. Only 3 treatments, the standard corrosive sublimate, the acid corrosive sublimate, and the corrosive sublimate and potassium iodide, were used on clean Irish Cobblers. An additional check of clean 1½-ounce whole tubers was included in this experiment. No Green Mountains were treated in 1933.

The chemically pure hydrochloric acid was compared with the commercial hydrochloric in the acidulated corrosive sublimate treatment to determine whether or not the pitting caused by this treatment might be due in part to impurities in the acid. Both treatments gave similar results, so that on the basis of this experiment one grade of acid cannot be considered better than the other.

Of the modifications of the acidulated corrosive sublimate treatment, none seemed to be superior to the original. It is of interest that the dip treatments in which the strength was slightly increased gave good results; but these combinations should be subjected to further trial, since rhizoctonia was not very severe in this experiment.

TABLE 12.—*Seed-potato treatment results—Irish Cobblers 1933*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over diseased nontreated check	
							Bushels	Approximate odds
Diseased, check, not treated		96.5	87	42	15	239.7 ± 6.9		
Diseased, corrosive sublimate 1–1000	1½ hrs.	99.6	91	0	0	250.9 ± 5.9	11.2 ± 9.1	1: 1
Diseased, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	99.6	90	8	1	251.7 ± 6.1	12.0 ± 9.2	2: 1
Diseased, corrosive sublimate 1–500 and 1% C.P. hydrochloric acid	3 min.	96.9	88	8	1	247.7 ± 4.0	8.0 ± 8.0	1: 1
Diseased, corrosive sublimate 1–1000 and 1% commercial hydrochloric acid	10 min.	95.4	86	6	3	253.3 ± 5.6	13.6 ± 8.9	2: 1
Diseased, corrosive sublimate 1–400 and 1% commercial hydrochloric acid	dip	97.3	92	0	3	251.7 ± 3.7	12.0 ± 7.8	2: 1
Diseased, corrosive sublimate 1–500 and 2% commercial hydrochloric acid	dip	98.1	90	4	3	254.9 ± 3.7	15.2 ± 7.8	4: 1
Diseased, corrosive sublimate 1–550 and 1% commercial hydrochloric acid	3 min.	98.8	90	4	1	257.3 ± 3.4	17.6 ± 7.7	7: 1
Diseased, corrosive sublimate 1–1200 and potassium iodide 1–400	dip	100	94	10	7	254.1 ± 5.7	14.4 ± 8.9	3: 1
Diseased, corrosive sublimate 1–1000 and potassium iodide 3–1000	dip	100	95	15	4	265.7 ± 4.7	26.0 ± 8.3	26: 1
Diseased, corrosive sublimate 1–1200 and potassium iodide 1–400, 5th time used	dip	100	94	12	3	249.3 ± 4.1	9.6 ± 8.0	1: 1
Diseased, corrosive sublimate 1–1200 and potassium iodide 1–400, 10th time used	dip	98.8	95	4	2	254.1 ± 3.3	14.4 ± 7.6	4: 1
Clean, check, not treated		100	97	0	0	250.5 ± 4.5	10.8 ± 8.2	2: 1
Clean, corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	99.2	94	0	1	258.1 ± 5.1	18.4 ± 8.6	5: 1

TABLE 13.—*Seed-potato treatment results—clean Irish Cobblers 1933*

Treatment		Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers		
						Yield per acre in bushels	Increase over non-treated check	
							Bushels	Approximate odds
Check, not treated		99.6	96	2	0	245.3 ± 6.2	.....	.....
Corrosive sublimate 1–1000	1½ hrs.	98.5	92	0	1	237.7 ± 7.1	−7.6 ± 9.4	0
Corrosive sublimate 1–500 and 1% commercial hydrochloric acid	3 min.	96.5	86	0		239.3 ± 5.8	−6.0 ± 8.5	0
Corrosive sublimate 1–1200 and potassium iodide 1–400	dip	98.8	96	0		241.3 ± 8.4	−4.0 ± 10.4	0
1½-oz. whole tubers, check, not treated		100	98	0		268.5 ± 8.8	23.2 ± 10.8	5:1

The relative vigor of the plants from tubers treated with corrosive sublimate in combination with potassium iodide was slightly higher than that of plants from tubers treated with the standard corrosive sublimate or with the acid corrosive sublimate mixtures.

In order to get information on the rate of loss of strength of the corrosive sublimate and potassium iodide treatment 1 lot was planted that was the first one dipped in a fresh solution, another one planted was the fifth, and another the tenth, starting with a little more solution than that necessary to cover a given lot of tubers. The last lot treated showed the best control, so there was no indication of loss of strength in these tests.

The yields in 1933 were low because of drought. The percentage of tubers bearing rhizoctonia sclerotia at harvest time was low also.

All of the treatments of the rhizoctonia-infected tubers gave increases in yield, but the increases were not significant, except in the case where a solution of corrosive sublimate 1–1000 and potassium iodide 3–1000 was used.

All of the treated lots of the clean Irish Cobblers yielded less than the nontreated control, but the differences were not significant.

DISCUSSION AND CONCLUSION

Considerable variation from year to year was experienced in these seed-potato treatment experiments. Such variation is to be expected since it is known that variations in soil moisture and temperature have a marked influence on the organism as well as on the host. The fact that the seed pieces were covered to a depth of about 6 inches in 1929, 1930, and 1931 and to a depth of about 4½ inches in 1932 and 1933 may have caused some of the differences recorded, since it is known that deep covering favors rhizoctonia. The differences recorded with the cold formaldehyde 1–120 used for 3 minutes are difficult to explain. This treatment was not expected to give good control, but was included for comparison with the 3-minute treatment with corrosive sublimate. In 1929 it gave satisfactory control of rhizoctonia but in 1931 and 1932 it was almost a complete failure in this regard. The only differences in the treatment in 1929, as compared with this treatment of the other years, were that the tubers were not given a 1-hour covering after treatment and that the temperature of the solution was slightly higher.

Seed-piece decay was not a factor in these experiments from 1929 to 1933. It is probable that handling the potatoes in bushel crates, that allowed the passage of air after treatment and cutting, contributed to these good results. The amount of benefit that might result from the treatment of seed, that must be held after cutting for long periods under less favorable conditions, has not been determined. It is known that seed-piece decay sometimes is an important factor in potato production and that in such instances seed tuber treatment is beneficial (7).

Notes were taken on the severity of stem lesions and on the degree of tuber infection. Since these figures, in gneral, corresponded closely with those for the percentage of stem lesions and the percentage of tubers infected, respectively, they were not included in the tables. In other words, where the percentage of stem lesions was high, the average lesion was more severe than where the percentage of stem lesions was low; likewise, where the percentage of infected tubers was high, the area covered by sclerotia usually was high.

The standard corrosive sublimate treatment controlled rhizoctonia satisfactorily without seriously injuring the tubers, but it has the disadvantage of being a long-time soak.

The acid corrosive sublimate treatment (corrosive sublimate 1–500 and 1 per cent commercial hydrochloric acid) used as a 3-minute soak, compared favorably with the standard corrosive sublimate treatment in control of rhizoctonia, but in nearly all instances it caused more pitting of the treated surface of the tubers. This treatment, as used by a few Maine growers in 1933, resulted in severe pitting of the eyes of the tubers and loss of stand in some instances. It is known that some of these growers did not dry the tubers properly after treatment. Whether other factors were involved has not been determined. In our experiments we have not experienced this severe pitting of the eyes, but we did get what proved to be a significant decrease in yield on clean Irish Cobblers in one experiment. At least, results indicate that this treatment should be used with caution.

The acid (hydrochloric or acetic) corrosive sublimate treatments were much more effective than corrosive sublimate of the same strength, without the acid, used for the same length of time. Glacial acetic acid first was used as 0.8 per cent with corrosive sublimate 1–500, but when it was compared with the regular acidulated corrosive sublimate treatment, using 1 per cent commercial hydrochloric acid, it was used at 0.56 per cent, since that strength gave about the same acidity, as determined by titrating against an alkali, as 1 per cent commercial hydrochloric acid. The acetic acid treatment as used was not as effective in the control of rhizoctonia to the commercial hydrochloric acid treatment. Also it did not pit the tubers as badly. Under certain conditions a blue mold grew on the tubers treated with the acetic acid mixture. This did not result in any noticeable effect but it was not observed where the hydrochloric acid was used.

The yellow mercuric oxide treatment, as used at 1–100, caused what might be considered a burning of the cut surface of the tubers when they came in contact with the treated surface after the tubers were cut. Also this treatment appeared to have a marked retarding effect on the early growth of the plants in some instances. Its control of rhizoctonia was very good. A more dilute mixture might have given more favorable results. In New York, yellow mercuric oxide was tried at 1–100 as a dip (2) and later was recom-

mended at the rate of 1 pound of yellow mercuric oxide to 15 gallons of water (8).

Laboratory tests indicated that a solution of corrosive sublimate 1–1200 and potassium iodide 1–400 used as a dip treatment (6) was effective in the control of the growth of rhizoctonia sclerotia. Field tests reported here, in general, substantiate these results. The high cost of the potassium iodide is a disadvantage of this treatment. It is well known that when potassium iodide is added to a solution of corrosive sublimate a yellow, then red, precipitate of mercuric iodide is formed that in turn is soluble in an excess of potassium iodide. It is this final solution that was used in these experiments, and, in order to get it, the ratio of potassium iodide to corrosive sublimate must be, at least, about 3 to 1.

The organic mercuries, Sanoseed and New Improved Semesan Bel, as used in these experiments, were fairly effective in the control of rhizoctonia. They have the advantage of being dip treatments.

Seed potatoes, selected as clean, when planted in a separate experiment, either treated or nontreated, over a period of 5 years, never produced a crop having over 7 per cent of the tubers infected with rhizoctonia. In most instances only 1 or 2 per cent of the tubers showed any sclerotia, even when washed. These results indicate that, under the conditions of these experiments, a program of selection and treatment would be more desirable than one of treatment alone, especially if the crop is to be used for seed purposes. The inclusion of selection in the control program is made even more desirable by the fact that none of the present treatments give perfect control of rhizoctonia without chance of injury to the potatoes.

The effect of crop rotation on rhizoctonia has not been determined under Maine conditions. All of these experiments were conducted on land that was in a 3-year rotation of oats, clover, and potatoes.

SUMMARY

The results of seed-potato treatment experiments for the years 1929 to 1933 are recorded.

In general, the plans of the experiments were similar to those of experiments conducted in Maine in 1927 and 1928.

Seed-tuber treatment of rhizoctonia-infected Irish Cobblers usually increased the stand (the percentage of plants that emerged), increased the average vigor of the plants, reduced the injury from stem lesions, reduced the percentage of infected tubers, and increased the yield.

Seed-tuber treatment of clean Irish Cobblers and field-run Green Mountains did not increase the yield. Tuber treatment of this type of potatoes might be regarded as an insurance against rhizoctonia that might develop over a period of years if the strain of potatoes is planted continuously with-

out selection and as an insurance against seed-piece decay, which occurs under some conditions.

Seed-piece decay was not a factor in these experiments.

The standard corrosive sublimate, 1½-hour soak, treatment was one of the most effective treatments used from the standpoint of rhizoctonia control.

The acidulated corrosive sublimate (corrosive sublimate 1–500 with 1 per cent commercial hydrochloric acid) 3-minute treatment, was satisfactory regarding rhizoctonia control but it seemed to be somewhat more dangerous from the standpoint of injury than the standard corrosive sublimate treatment. Special care should be taken that tubers are dried after treatment.

Yellow mercuric oxide 1–100, used as a dip, gave good control of rhizoctonia but was injurious in some instances.

A solution of corrosive sublimate 1–1200 and potassium iodide 1–400, used as a dip treatment, gave promising results in these experiments.

The organic mercury dips, Sanoseed and New Improved Semesan Bel, proved to be superior to organic mercury dips sold in previous years. As used they were slightly inferior to the standard corrosive sublimate treatment in regard to rhizoctonia control.

The term "dip treatment," as used, indicates that the tubers were dipped 3 times in rapid succession or that they were placed in a solution, agitated slightly, and then removed. In either case thorough wetting resulted.

Clean Irish Cobblers, when planted in a separate experiment, either treated or not, produced a crop bearing very few rhizoctonia sclerotia.

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THE EFFECT OF SEVERAL COLLECTIONS OF TILLETIA TRITICI AND T. LEVIS ON THE MORPHOLOGY OF SPRING WHEATS[1]

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INTRODUCTION

In the course of bunt studies conducted at the University of Alberta, Edmonton, Canada, striking morphological differences were observed in wheat plants infected with Tilletia. The most striking of the effects observed were: the reduction in culm length, elongation of the spike, and the shape of the bunt ball. Since there appeared to exist differences among the wheats grown in the bunt test for the above characters, it was considered desirable to secure more definite information on the influence of different collections of bunt on several varieties for these morphological changes. Other workers have reported morphological changes in wheat resulting from infection by the bunt fungi. The natures of these reports are somewhat conflicting.

MATERIALS AND METHODS

The material was grown at the University of Alberta in 1931. Six varieties of spring wheat: Reward, C.A.N.[3] 1509, Little Club, C.A.N. 1620, Pentad, C.A.N. 1633, Hope, C.A.N. 1615, Garnet, C.A.N. 1316 and Kota, C.A.N. 1364, were used as hosts. The seed of the 6 varieties was inoculated separately with chlamydospores from five collections of bunt. The collections of bunt consisted of one of *Tilletia tritici* (Bjerk.) Wint. obtained from Garnet grown at Egremont, Alberta; and 4 of *T. levis* (Kühn), which were obtained from Garnet, Marquis, Reward, and H-44, grown in the experimental plots at the University of Alberta in 1930. These collections have been designated by the numbers 1, 2, 3, 4, and 5, respectively.

The method of inoculation consisted of applying approximately equal amounts of dry chlamydospores to the seed, in an envelope. The envelope containing the mixture of seed and spores was then shaken thoroughly. Precautions were taken to avoid mixing of the inoculum in the preparation of the several samples and in the sowing. Hands and equipment were thoroughly washed in alcohol between handlings of the different lots of

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material. The inoculated seed was sown in duplicate at the rate of 100 seeds to a 10-foot row. Noninoculated seed of each variety was sown as a check. At maturity the plants from the duplicate rows were pulled and bulked.

Certain of the inoculated plants showed no infection, others were partially infected, and still others completely so. As far as the material permitted, 20 plants for each of these 3 classes of plants from inoculated seed were selected at random. The material was studied in detail to find the reaction of the several wheat varieties to the different collections of bunt in regard to culm length, spike elongation, bunt-ball shape, and any possible interrelationships between these characters.

The culm length was measured in centimeters from the crown of the plant to the base of the spike for each culm of every plant studied. The spikes were classified into 7 classes for spike elongation. The classes ranged from 1, indicating a shortening of the spike, to 7, indicating considerable elongation. The bunt balls were classified into 5 classes from 1 to 5 on the basis of their length in proportion to width, those in class 1 being round, while those in class 5 were long and narrow. A statistical analysis of the data was made after the completion of the preliminary notes.

CULM LENGTH

Although the effect of bunt on the culm length of the wheat plant has been observed by a large number of workers, very little comprehensive work has been published. Harwood (5), in Michigan, observed a difference in the height of wheat plants infected with *Tilletia levis* and *T. tritici*. He states that the culms of wheat infected with *T. tritici* (low smut) grew only to a height of 1 to 1.5 feet, while those infected with *T. levis* (high smut) were little if any shorter than the culms of noninfected plants. Potter and Coons (8) confirmed Harwood's observations, stating that the culms of wheat plants infected with *T. levis* were from 2 to 4 inches shorter, while those infected with *T. tritici* were 12 inches shorter than the culms of noninfected plants. They also report that plants partially infected with *T. tritici* were taller than completely infected plants. Bressman (2) studied ten wheat varieties, each inoculated separately with 43 different collections of bunt. He concludes that, in general, the culms of plants infected with *T. tritici* are shorter than those infected with *T. levis* and that in both the culms are shorter than those of noninfected plants. However, he states that this difference in culm length is not a constant characteristic of the species.

Other investigators report no difference in the culm length of wheat infected with the 2 species of Tilletia. Selby (10), in Ohio, found no difference in the relative degree of stunting of wheat infected with *T. levis* or *T. tritici*. McAlpine (7), in Australia, arrived at similar conclusions.

Rodenhiser (9) demonstrated that both *T. levis* and *T. tritici* may cause significant reductions in the culm length of the wheat plant. He states that whether or not significant differences occur in the relative degree of stunting caused by the 2 species of Tilletia depends on the physiologic form with which the plant is infected. He suggests that it is entirely possible that the physiologic forms tested might have reacted differently on other varieties and under different environmental conditions.

A number of workers have studied the effect of one or the other species of Tilletia on the culm length of wheat plants. Barrus (1) observed a shortening of the culms of diseased plants, as compared with those of normal plants, when infected with *T. levis*. This, he states, is due to a reduction in length of all the internodes. Woolman and Humphrey (15) report a greater shortening of the culms of *Triticum compactum* as compared with those of *T. vulgare*, when attacked with bunt. Vilkaitis (13) found that *Tilletia tritici* reduces the culm length of an unspecified wheat by as much as 25 per cent. Viennot (12) reports that the reduction of height of Bon Fermier wheat infected with *T. tritici* is due to a decrease in the number of internodes more than to a shortening between the internodes.

EXPERIMENTAL RESULTS

The plants studied were placed in 4 groups as follows: nonbunted plants from the noninoculated seed; nonbunted plants from inoculated seed; and partially bunted and completely bunted plants from inoculated seed. The nonbunted and bunted culms of partially bunted plants were treated separately. The data given in table 1 show the effect of the several collections of bunt on the culm length of the 6 wheat varieties for the different groupings. The data for the differences among and significance for the various groupings are given in table 2. The method employed in calculating the significance of the differences among the various means was Fisher's (4) method of obtaining the significance of differences among the means of small samples that are not paired in any way. The value of P was obtained from Fisher's Table of "t."

An examination of the data in tables 1 and 2 reveals many interesting observations. The difference in culm length of plants grown from noninoculated seed and nonbunted plants grown from inoculated seed, is significant in 11 cases out of 17. The differences are significant for Reward, when inoculated with collections 1 and 2, but not with collections 3 and 5; for Little Club, with 1, 2, and 3, but not with 4; for Pentad, with 2, 3, and 4, but not with 1; for Hope, with 1 but not with 2; and Kota, with 1 and 2 but not with 5. These data indicate that almost all plants become infected when inoculated, and that resistance as ordinarily used is the suppression of the fungus sufficiently so that bunted grain is not produced, rather than

the ability to inhibit infection, though the latter may also be a form of resistance. Infection is evidenced by the shortened culms of nonbunted plants grown from inoculated seed. A detailed histological study would be necessary to verify this evidence.

A comparison of the culm length between the nonbunted culms of nonbunted and partially bunted inoculated plants shows that in 6 cases out of 19 the differences are significant. In 2 of the 6 cases where the differences were significant, the nonbunted culms of partially bunted plants were the longest. No satisfactory reason can be offered for the occurrence of these significant differences, especially when, in certain cases, the culms of nonbunted inoculated plants are the longer; while in other cases the nonbunted culms of partially bunted plants are the longer. The lack of significant differences in most of the cases and the fact that, where the differences are significant, they are not uniformly in one direction, lends support to the hypothesis that nearly all plants become infected when inoculated.

Several comparisons were used in studying the differences in culm length between nonbunted and bunted culms, namely, noninoculated and completely bunted plants; nonbunted inoculated and bunted culms of partially bunted plants; nonbunted inoculated and completely bunted plants, nonbunted culms of partially bunted plants and the culms of completely bunted plants; and nonbunted and bunted culms of partially bunted plants. In every comparison the difference was significant, save with Little Club inoculated with collections 1, 2, 3, and 4 for the nonbunted and bunted culms of partially bunted plants. The culm length of nonbunted inoculated plants for Little Club with all collections, were longer than the nonbunted culms of partially infected plants, and were significant in 3 out of 4 cases. Also, it is interesting to note that, for Little Club, with all collections, the bunted culms of partially infected plants were longer than the bunted culms of completely infected plants, and that in 3 out of the 4 cases the difference was significant. No definite reason can be given for this apparent peculiar behavior of Little Club.

A comparison of the bunted culms of partially bunted plants and the culms of completely bunted plants shows that in all cases the bunted culms of partially bunted plants are the taller. However, the differences are only significant in 4 out of 15 comparisons, namely, collections 1 on Reward and 1, 2 and 3 on Little Club.

The data clearly indicate that infection of a wheat plant with either *Tilletia levis* or *T. tritici* may cause a significant reduction in culm length. In the degree of stunting the various collections appear to act somewhat differently on the several wheat varieties. This is shown by the reaction of Reward, Little Club and Pentad in culm length when infected with collections 1, 2 and 3. A much greater reduction resulted in the mean culm

TABLE 1.—*Effect of several collections of bunt on the culm length, in centimeters, of six wheat varieties*

Variety	Bunt collection				
	1	2	3	4	5
REWARD					
Seed not inoculated					
Plants nonbunted	.....	.....	96.6 ± 1.39[a]	.....	.....
Seed inoculated					
Plants nonbunted	90.8 ± 1.39	85.6 ± 1.45	95.5 ± 1.29	.....	95.8 ± 1.10
Plants partially bunted:					
Nonbunted culms	93.3 ± 1.39	91.1 ± 2.15	95.6 ± 1.60	.....	97.6 ± 2.45
Bunted culms	82.7 ± 0.98	81.1 ± 1.56	88.2 ± 1.32	.....	88.0 ± 1.41
Plants completely bunted	77.9 ± 1.16	78.5 ± 1.13	86.6 ± 1.20	.....	87.7 ± 1.10
LITTLE CLUB					
Seed not inoculated					
Plants nonbunted	.....	.....	101.0 ± 1.54	.....	.....
Seed inoculated					
Plants nonbunted	86.1 ± 1.05	94.9 ± 1.10	91.7 ± 1.29	100.0 ± 1.08	.....
Plants partially bunted:					
Nonbunted culms	84.7 ± 1.72	90.6 ± 1.54	87.5 ± 0.95	93.1 ± 1.70	.....
Bunted culms	80.5 ± 1.59	86.6 ± 1.69	86.1 ± 1.59	88.5 ± 2.06	.....
Plants completely bunted	74.0 ± 1.19	81.5 ± 1.29	81.6 ± 1.22	86.4 ± 1.42	.....
PENTAD					
Seed not inoculated					
Plants nonbunted	.....	.....	110.3 ± 1.25	.....	.....
Seed inoculated					
Plants nonbunted	110.9 ± 1.38	100.2 ± 1.51	105.6 ± 1.20	103.3 ± 1.30	.....
Plants partially bunted:					
Nonbunted culms	105.2 ± 1.75	100.7 ± 2.19	104.1 ± 2.06	103.8 ± 1.93	.....
Bunted culms	84.6 ± 2.36	79.3 ± 2.74	86.1 ± 2.42	84.1 ± 1.99	.....
Plants completely bunted	.....	77.0 ± 2.82	.....	80.2 ± 2.34	.....

TABLE 1.—(*Continued*)

Variety	Bunt collection				
	1	2	3	4	5
HOPE					
Seed not inoculated					
Plants nonbunted			96.6 ± 1.59		
Seed inoculated					
Plants nonbunted	92.2 ± 1.54	96.9 ± 1.17			
Plants partially bunted:					
Nonbunted culms	93.3 ± 1.10	93.9 ± 2.37			
Bunted culms	84.0 ± 1.19	84.7 ± 3.38			
Plants completely bunted					
KOTA					
Seed not inoculated					
Plants nonbunted			111.5 ± 1.20		
Seed inoculated					
Plants nonbunted	102.6 ± 2.30	95.2 ± 1.11			112.1 ± 1.54
Plants partially bunted:					
Nonbunted culms	101.3 ± 1.72	92.5 ± 2.22			117.3 ± 2.02
Bunted culms	89.3 ± 1.57	85.4 ± 1.48			101.9 ± 1.44
Plants completely bunted	87.5 ± 1.17	82.7 ± 1.50			99.8 ± 1.48
GARNET					
Seed not inoculated					
Plants nonbunted					
Seed inoculated					
Plants nonbunted	99.7 ± 0.83			97.2 .... 0.62	98.4 ± 0.96
Plants partially bunted:					
Nonbunted culms	97.7 ± 1.38			96.7 ± 1.41	95.7 ± 1.41
Bunted culms	87.7 ± 1.62			87.2 ± 1.78	91.9 ± 1.14
Plants completely bunted					87.0 ± 2.37

[a] Mean with standard error.

length for Reward infected with collections 1 and 2 than when infected with collection 3; Little Club, infected with collection 1 than with collections 2 and 3; and Pentad, infected with collection 2 than with collections 1 and 3.

No consistent evidence was obtained to lend support to the opinion that the culms of wheat plants infected with *Tilletia tritici* are shorter than those infected with *T. levis*, which is in agreement with the results reported by Selby (10), McAlpine (7), and Rodenhiser (9); but not with those of Harwood (5) and Potter and Coons (8). The evidence presented lends support to the statement of Rodenhiser (9) to the effect that, whether or not there is a significant difference in the relative degree of stunting caused by the two species of Tilletia, depends upon the physiologic form of Tilletia with which the plants are infected.

TABLE 2.—*Differences in average culm length in centimeters between various groupings of six wheat varieties inoculated separately with several collections of bunt*

Variety	Bunt collection				
	1	2	3	4	5
REWARD					
1. Nonbunted plants, seed not inoculated and-inoculated	5.8 ± 1.9[a]	10.6 ± 1.9[a]	0.7 ± 1.9	.....	0.4 ± 1.8
2. Nonbunted culms of nonbunted and partially bunted inoculated plants	2.5 ± 1.9	5.5 ± 2.2[a]	0.1 ± 1.8	.....	1.8 ± 2.2
3. Noninoculated plants and completely bunted plants	18.7 ± 1.8[a]	17.7 ± 1.8[a]	9.6 ± 1.8[a]	.....	8.5 ± 1.8[a]
4. Nonbunted plants and bunted culms, partially bunted inoculated plants	8.1 ± 1.6[a]	4.5 ± 2.1[a]	7.3 ± 1.9[a]	.....	7.8 ± 1.8[a]
5. Nonbunted and completely bunted inoculated plants	12.9 ± 1.8[a]	6.9 ± 1.8[a]	8.8 ± 1.8[a]	.....	8.1 ± 1.6[a]
6. Nonbunted and bunted culms of partially bunted plants	10.6 ± 1.8[a]	9.0 ± 2.7[a]	7.4 ± 2.1[a]	.....	9.6 ± 2.8[a]
7. Nonbunted culms partially bunted plants and completely bunted plants	15.3 ± 1.8[a]	12.6 ± 2.4[a]	9.0 ± 2.1[a]	.....	9.9 ± 2.7[a]
8. Bunted culms of partially and completely bunted plants	4.9 ± 1.5[a]	2.6 ± 1.9	1.6 ± 1.8	.....	0.3 ± 1.9

TABLE 2.—(*Continued*)

Variety	Bunt collection				
	1	2	3	4	5
LITTLE CLUB[b]					
1	14.9 ± 1.9[a]	5.1 ± 1.9[a]	9.3 ± 2.1[a]	1.0 ± 1.9[a]	.....
2	1.9 ± 2.2	4.3 ± 1.9[a]	4.2 ± 1.6[a]	6.9 ± 2.1[a]	.....
3	27.0 ± 1.9[a]	19.5 ± 1.9[a]	19.4 ± 1.9[a]	14.6 ± 2.1[a]	.....
4	5.6 ± 1.9[a]	8.3 ± 2.1[a]	5.6 ± 2.1[a]	11.5 ± 2.4[a]	.....
5	12.1 ± 1.6[a]	13.4 ± 1.6[a]	10.1 ± 1.8[a]	13.6 ± 1.8[a]	.....
6	4.2 ± 2.4	4.0 ± 2.4	1.4 ± 1.9	4.7 ± 2.7	.....
7	10.8 ± 2.1[a]	9.1 ± 1.9[a]	5.9 ± 1.5[a]	6.7 ± 2.2[a]	.....
8	6.6 ± 2.1[a]	5.1 ± 2.1[a]	4.5 ± 1.9[a]	2.1 ± 2.5[a]	.....
PENTAD					
1	0.6 ± 1.9	10.1 ± 1.9[a]	4.7 ± 1.8[a]	7.0 ± 1.8[a]	.....
2	5.7 ± 2.2[a]	0.5 ± 2.7	1.5 ± 2.4	0.5 ± 2.4	.....
3	.....	33.3 ± 3.1[a]	.....	30.1 ± 2.7[a]	.....
4	26.3 ± 2.7[a]	20.9 ± 3.1[a]	19.5 ± 2.7[a]	19.2 ± 2.4[a]	.....
5	.....	23.2 ± 3.2[a]	.....	23.1 ± 2.7[a]	.....
6	20.6 ± 3.0[a]	21.4 ± 3.5[a]	18.0 ± 2.9[a]	19.7 ± 2.9[a]	.....
7	.....	23.7 ± 3.5[a]	.....	23.6 ± 3.0[a]	.....
8	.....	2.3 ± 3.8	.....	3.9 ± 3.1	.....
HOPE					
1	4.4 ± 2.1[a]	0.3 ± 2.1	.....	.....	.....
2	1.1 ± 1.9	3.0 ± 2.7	.....	.....	.....
3	.....	.....	.....	.....	.....
4	9.3 ± 1.6[a]	12.2 ± 3.5[a]	.....	.....	.....
5	.....	.....	.....	.....	.....
6	8.2 ± 1.9[a]	9.2 ± 4.1[a]	.....	.....	.....
7	.....	.....	.....	.....	.....
8	.....	.....	.....	.....	.....
KOTA					
1	8.9 ± 2.7[a]	16.3 ± 1.6[a]	.....	.....	0.6 ± 1.9
2	1.3 ± 2.8	2.7 ± 2.5	.....	.....	5.2 ± 2.1[a]
3	24.0 ± 1.6[a]	28.8 ± 1.9[a]	.....	.....	11.7 ± 1.9[a]
4	13.3 ± 2.8[a]	9.8 ± 1.8[a]	.....	.....	10.2 ± 2.1[a]
5	15.1 ± 2.5[a]	12.5 ± 1.8[a]	.....	.....	12.3 ± 1.5[a]
6	12.0 ± 2.4[a]	7.1 ± 2.7[a]	.....	.....	15.4 ± 2.5[a]
7	13.8 ± 2.1[a]	9.8 ± 2.2[a]	.....	.....	17.5 ± 2.5[a]
8	1.8 ± 1.9	2.7 ± 2.1	.....	.....	2.1 ± 2.1
GARNET					
1	.....	.....	.....	.....	.....
2	2.0 ± 1.6	.....	.....	0.5 ± 1.5	2.7 ± 1.9
3	.....	.....	.....	.....	.....
4	12.0 ± 1.8[a]	.....	.....	9.7 ± 1.4[a]	6.5 ± 1.5[a]
5	.....	.....	.....	10.0 ± 1.9[a]	11.4 ± 2.5[a]
6	10.0 ± 2.1[a]	.....	.....	8.2 ± 1.9[a]	3.8 ± 1.8[a]
7	.....	.....	.....	9.5 ± 2.2[a]	8.7 ± 2.7[a]
8	.....	.....	.....	2.3 ± 2.2	4.9 ± 2.7

[a] P value less than 0.05.
[b] Numbers refer to enumerated designations in column 1 of table 2.

SPIKE ELONGATION

Heald and Woolman (6) observed that the heads of club varieties of wheat affected with bunt are more slender in form than are normal heads; while those of the Fife or Bluestem varieties do not show such a noticeable change. In the latter, the infected heads have a more loose or open appearance than normal heads, due to a divergence of the glumes caused by an enlargement of the smutted kernels. Barrus (1) observed that the heads infected with *Tilletia levis* of a variety of *Triticum vulgare*, similar to Dawson's Golden Chaff, were shorter and slimmer than those from normal plants. Potter and Coons (8), 1918, state that the heads of wheat plants infected with *Tilletia tritici* are enlarged and present a more crowded appearance than do normal heads; while those infected with *T. levis* are more slender and open in appearance. Woolman and Humphrey (15) report that bunted heads of *Triticum compactum* wheats are much elongated, approaching those of the *T. vulgare* group in form. However, there are frequent exceptions, particularly among the spring-sown clubs. Partially infected heads, they found, were more normal in size and shape than completely infected heads.

Weston (14) reports that winter-sown American Club wheat, infected with bunt, produces much-elongated heads, which resemble those of the *Triticum vulgare* group. He states that such marked elongation was not noted in the species of wheat under observation; *viz.*, *T. sphaerococcum* Perc., *T. spelta* L., *T. polonicum* L., *T. durum* Desf., *T. dicoccum* Schr., *T. persicum* Vav. and *T. turgidum* L. He cites the works of Appel and Edler, both of whom report an elongation of the head in spring-sown club wheats, when affected with bunt. Vilkaitis (13) found a shortening of the head of an unspecified wheat by 15 per cent when infected with *T. tritici*. Rodenhiser (9) observed differences in host reaction for the general shape of heads affected with bunt, but states that these differences were not constant for a single species of Tilletia. Bressman (2) obtained an elongation of the spike in the club varieties Albit and Hybrid 128, when affected with bunt. Smith (11) studied the reaction of Martin wheat to different physiologic forms of *T. tritici*. He found that heads on plants infected with physiologic form T_2 were more lax and narrower than those infected with form T_3.

EXPERIMENTAL RESULTS

The infected spikes were classified into seven classes for the degree of spike elongation, namely: 1 indicating a slight shortening, 2 indicating no elongation, and 3 to 7 indicating elongation of various degrees. The data in table 3 give the effect of bunt infection on the spike length for both the partially and completely infected plants of the six wheat varieties, inoculated separately, with the different bunt collections. An analysis of these data shows that spike elongation, resulting from bunt infection, occurred

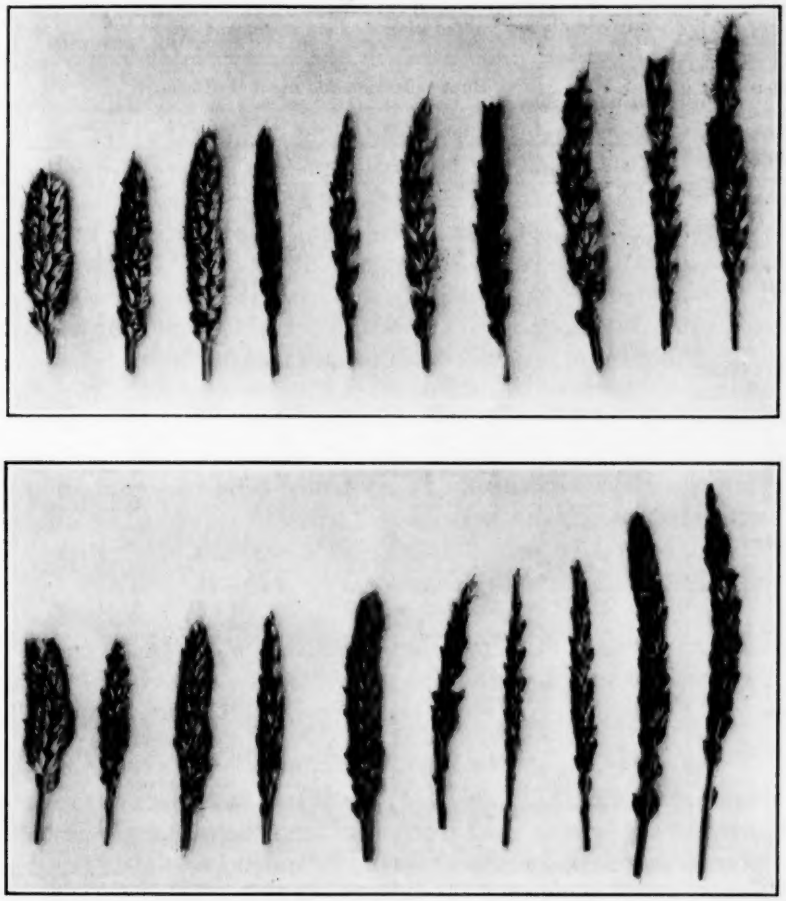

FIG. 1. Extent and great degree of variability in spike elongation, found in infected heads of Little Club for collection 1 of *Tilletia tritici* (upper) and collection 2 of *T. levis*, both of which were obtained from Garnet (lower).

only in the more susceptible varieties of Reward, Little Club and Kota; while in the case of the more resistant varieties of Pentad, Hope and Garnet, little or no spike elongation was found. The susceptible varieties reacted somewhat differently to the several collections of bunt fungi used. Little Club showed considerable elongation of the spike for all collections. In figure 1 are shown heads of Little Club wheat both normal and infected with collection 1 (*T. tritici*) and collection 2 (*T. levis*), both obtained from Garnet. This figure illustrates both the extent of, and the great degree of variability in, the spike elongation found among the heads of Little Club wheat infected separately with both species of Tilletia. Little or no elonga-

TABLE 3.—*The effect of the several collections of bunt on the length of infected heads of partially and completely bunted plants of six wheat varieties*

Variety and classes for spike elongation	Bunt collection and number of heads									
	1		2		3		4		5	
	P[a]	C[b]	P	C	P	C	P	C	P	C
Reward										
1[c]	....	....	1	1	....	....	....	....	2	....
2	50	57	25	24	23	31	....	....	43	38
3	3	9	17	18	18	22	....	....	6	9
4	....	....	7	6	4	8	....	....	....	....
5	....	....	....	2	3	1	....	....	....	....
6	....	....	....	1	....	....	....	....	....	....
7	....	....	....	....	....	....	....	....	....	....
Little Club										
1	....	....	....	2	1	2	....	....	....	....
2	17	31	13	20	9	24	6	11	....	....
3	22	21	15	18	23	35	13	18	....	....
4	10	25	5	7	16	17	14	26	....	....
5	8	3	3	2	2	5	6	7	....	....
6	2	....	1	2	....	1	....	1	....	....
7	....	....	2	....	....	....	....	....	....	....
Pentad										
1	....	....	....	....	....	....	....	....	....	....
2	33	....	33	18	23	6	33	20	....	....
3	....	....	3	2	9	....	8	3	....	....
4	....	....	....	....	....	....	....	....	....	....
5	....	....	....	....	....	....	....	....	....	....
6	....	....	....	....	....	....	....	....	....	....
7	....	....	....	....	....	....	....	....	....	....
Hope										
1	....	....	....	....	....	....	....	....	....	....
2	14	....	20	....	....	....	....	....	....	....
3	....	....	....	....	....	....	....	....	....	....
4	....	....	....	....	....	....	....	....	....	....
5	....	....	....	....	....	....	....	....	....	....
6	....	....	....	....	....	....	....	....	....	....
7	....	....	....	....	....	....	....	....	....	....
Kota										
1	....	1	2	2	....	....	....	....	3	3
2	39	56	22	34	....	....	....	....	34	46
3	5	3	14	17	....	....	....	....	7	9
4	1	....	4	4	....	....	....	....	4	2
5	....	....	1	1	....	....	....	....	1	....
6	....	....	....	....	....	....	....	....	....	....
7	....	....	....	....	....	....	....	....	....	....
Garnet										
1	....	....	....	....	....	....	....	....	....	....
2	30	6	....	....	....	....	40	18	28	13
3	1	1	....	....	....	....	....	1	9	3
4	....	....	....	....	....	....	....	....	2	....
5	....	....	....	....	....	....	....	....	....	....
6	....	....	....	....	....	....	....	....	....	....
7	....	....	....	....	....	....	....	....	....	....

[a] P = Bunted culms partially infected plants.
[b] C = Bunted culms completely infected plants.
[c] Numbers refer to enumerated designations in column 1, table 2.

tion occurred in the spikes of Reward infected with collections 1 and 5, but when infected with collections 2 and 3 considerable elongation resulted. Kota infected with collection 1 showed little or no spike elongation, but when infected with either collections 2 or 5, considerable spike elongation occurred. Infected spikes of partially infected plants showed as much spike elongation as the spikes of completely infected plants.

The marked elongation of the spike of Little Club, when infected with bunt, is in agreement with other results reported on club wheats for this character (2, 6, 14, 15). No indication of a consistent shortening of infected spikes, as reported by Barrus (1) and Vilkaitis (13) was obtained. Potter and Coons (8) report differences between the two species of Tilletia in regard to general shape of infected heads. In the present investigation differences were noted but were not found to be consistent for a single species, which agrees with the results of Rodenhiser (9). Smith (11) reports that heads of Martin wheat, infected with physiologic form T_2, were of a different shape than those infected with form T_3. In the present study indications were also obtained that different collections of bunt react differently on the same variety. In the case of Reward, infection with collections 2 and 3 resulted in a much greater degree of spike elongation than when infected with collections 1 and 5.

The results of this investigation indicate that the degree of spike elongation of infected heads is influenced by both the collection or physiologic form of bunt used, and the host.

SHAPE OF BUNT BALLS

Barrus (1) observed that the shape of bunt balls of infected heads were smaller in all dimensions than the kernels from normal heads. Potter and Coons (8) state that the sori of *Tilletia levis* are longer and narrower than those of *T. tritici*. Rodenhiser (9) reports differences in the general shape of bunt balls of the two species of Tilletia on different hosts, but these differences were not constant for a single species. Feucht (3) states that with both species of Tilletia he obtained swollen as well as slender, almost elongated, oval balls; and that their general shape seemed to be more determined by variety than by any inherent tendency of the fungus. Smith (11) found differences in the bunt ball shape of two physiologic forms of *T. tritici* on Martin wheat, one of which produced a shrivelled angular ball, while the ball of the other form was rounder in shape.

EXPERIMENTAL RESULTS

The bunt balls were classified into 5 classes for shape, 1 being round and 5 long and narrow (Fig. 2). In table 4 the data give the total length in centimeters of 10 kernels of the 6 varieties and the mean class for bunt-ball shape for each variety of wheat infected with each collection of bunt.

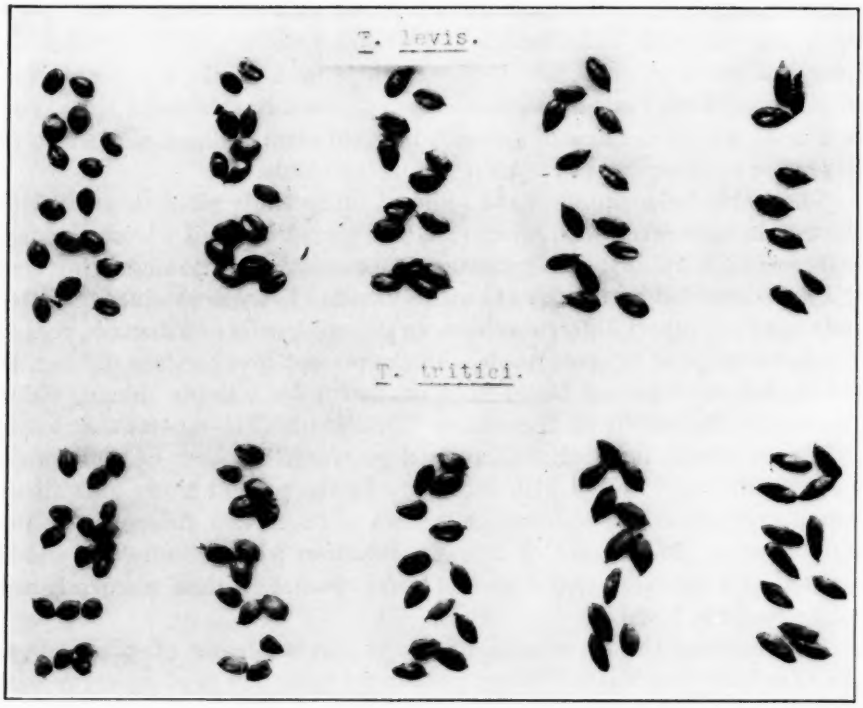

FIG. 2. Great variability found in the size and shape of the bunt balls of both species of Tilletia.

No difference was noticed in the bunt-ball shape of partially infected plants and those of completely infected plants for any variety infected with any collection. The bunt balls within a single spike showed considerable variation in shape and size. Little difference existed in the average bunt ball-shape for the different collections on any single variety.

The data in table 4 show that the average bunt-ball shape of collection 1, *Tilletia tritici*, tended to be rounder than those from the 4 collections of *T. levis*. This cannot be taken as conclusive evidence that the bunt balls of *T. tritici* tend to be smaller than those of *T. levis*, for only one collection of *T. tritici* was used. Since the range in shape of the bunt balls of *T. tritici* was similar to that for *T. levis* (Fig. 2), it is impossible to tell by the shape of a bunt ball to which of the two species it belongs. In figure 2 is shown the great variability found in the size and shape of the bunt balls of both species of Tilletia. The data show that the bunt balls on the varieties of Pentad, Hope and Kota having the long normal kernel, tended to be longer than those produced on the varieties of Reward, Little Club and Garnet, which have shorter normal kernels.

TABLE 4.—*Total length in centimeters of ten kernels from, and average bunt-ball shape for each bunt collection on Reward, Little Club, Pentad, Hope, Kota, and Garnet spring wheats*

Variety	Total length of 10 kernels in centimeters	Classes for average bunt ball shape[a] for the different collections of bunt				
		1	2	3	4	5
Reward	6.3	2	3	3	...	3
Little Club	6.1	2	2.5	2.5	2.5	...
Pentad	7.1	4	4	4.5	4	...
Hope	7.3	2.5	3.5	...	...	...
Kota	7.0	3	4	...	4	...
Garnet	6.1	1.5	...	...	2	2.5

[a] 1 = round to 5 = long and narrow.

That certain physiologic forms produce bunt balls of different shape is clearly indicated by the work of Smith (11), who obtained differences in bunt-ball shape between 2 physiologic forms of *Tilletia tritici*, on Martin wheat. There appears to be a general tendency for the shape of the bunt balls produced by *T. tritici* to be slightly rounder than those produced by *T. levis*, which was previously reported by Potter and Coons (8). However, this general tendency found in the present study cannot be taken as being conclusive, for only one collection of *T. tritici* was tested. As previously pointed out, it is impossible to distinguish between the bunt balls of the two species, for both have a similar range for size and shape. The results of this study strongly support the statement of Feucht (3) to the effect that the general shape of bunt balls is determined more by variety than by any inherent tendency of the fungus.

ASSOCIATION BETWEEN CHARACTERS

Tests for independence were calculated in order to determine any possible relationships that may exist between length of culm, spike elongation, and bunt-ball shape. The method of analysis used was that described by Fisher (4). Tests were calculated for both the partially and completely infected plants with each variety for all collections of bunt with which they were inoculated. Significant associations were obtained only in 3 instances: Reward, infected with collection 2, showed a positive association between spike elongation and bunt-ball shape, for the completely infected plants; Little Club, infected with collection 1, showed a positive association between length of culm and spike elongation, for completely infected culms; Little Club, infected with collection 1, also showed a positive association between

length of culm and bunt-ball shape. In these 3 instances no association was found in the case of the bunted culms of the partially infected plants. The data for the other varieties indicate that, in general, there are no associations between bunt-ball shape, spike elongation, or length of culm in the varieties tested, and for the collections of bunt used.

SUMMARY

The effect of bunt on the morphology of the wheat plant was studied with the 6 spring wheat varieties: Reward, Little Club, Pentad, Hope, Kota, and Garnet, inoculated separately with one collection of *Tilletia tritici* and four of *T. levis*.

Infection with either species of Tilletia resulted in a reduction of the culm length of the wheat plant. However, no significant difference was obtained in the degree of reduction produced by the two species.

The different collections reacted somewhat differently on the different wheat varieties.

The stunting of inoculated nonbunted plants indicates that nearly all plants became infected when inoculated and kept under favorable conditions for the fungus to develop.

In general, no difference was detected in the culm length of the bunted culms of partially bunted plants and those of the completely bunted plants.

Spike elongation of bunt-infected heads occurred only in the more susceptible varieties: Reward, Little Club, and Kota. The susceptible varieties tended to react somewhat differently to the different collections used.

The general shape of the bunt balls was determined more by the variety than by any inherent tendency of the fungus.

The range in size of the bunt balls for all collections was the same. However, those of a single collection of *T. tritici* tended on the average to be smaller and rounder than those of *T. levis*.

No general association was found to exist between culm length, spike elongation and bunt-ball shape.

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FACTOR RELATIONS IN WHEAT FOR RESISTANCE TO PUCCINIA GRAMINIS TRITICI, PUCCINIA GLUMARUM AND ERYSIPHE GRAMINIS[1]

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INTRODUCTION

Three decades have elapsed since Biffen (2, 3) first demonstrated that resistance to stripe rust of wheat was inherited according to Mendelian laws. During this time an enormous amount of information concerning the genetics of disease resistance in plants has been accumulated. Surprisingly little attention, however, has been devoted to the question of the genetic relationship between resistance to different diseases. Two causes, at least, underlie this neglect. In the first place, most of the work has been prompted by local needs. For a particular crop in any one locality there are commonly one or, perhaps, two diseases of outstanding importance. The most urgent problems have, not unnaturally, received the first attention. In the second place, most investigators in this field have sought to determine the actual number of genes concerned in the inheritance of the reaction to the disease in which they were interested. This necessitates the use of F_3 material, and the small amount of available seed usually limits the study to one, or at most two, diseases. A few cases of genetic linkage have been reported, and these will be briefly reviewed.

Hayes, Aamodt, and Stevenson (7) and Goulden and Neatby (4) have made certain observations that suggest a linkage between susceptibility to black chaff and resistance to *Puccinia graminis tritici* in wheat crosses. This relationship has been studied further by Hayes *et al.* (8) and Ausemus (1). It is not certain, in the opinion of the present writer, that the relationship is due to genetic linkage. It may be that rust-infected plants do not provide a suitable medium for the development of black chaff, and that in the absence of rust infection they might be susceptible to black chaff. This possibility has not been adequately considered.

Hayes *et al.* (8) found some indication of linkage between resistance to *Puccinia triticina* and resistance to *P. graminis tritici.*

In a cross between the wheat varieties Federation and Thew, Waterhouse (13) found resistance to *Puccinia triticina* and resistance to *Erysiphe graminis* to be completely linked.

[1] Contribution from the National Research Council (U. S. A.) and the Dominion Department of Agriculture, Canada.

[2] Fellow of the National Research Council.

In a study of the inheritance of resistance to bunt of wheat in the cross Kota × Garnet, Kilduff (9) found resistance to *Tilletia laevis* (*T. foetens*) to be correlated with resistance to *T. tritici*.

Reed (12) made a study of the inheritance of resistance to *Ustilago avenae* and *U. levis* in crosses between the oat varieties Black Mesdag and Hulless. The results indicated that the resistance of Black Mesdag to the two smuts was inherited as a simple Mendelian dominant. Welsh (14) also found resistance to *U. avenae* and to *U. levis* to be closely correlated in the cross Victory × (Minota-White Russian × Black Mesdag).

Several cases of independent inheritance of the reaction to different plant diseases have been reported. These will not be reviewed here. A list of references is given by Hansen (6).

The following pages contain a report of the preliminary results of a study of the genetic relationship between the reactions to various fungous diseases of wheat.

MATERIALS

The present study is concerned with the genetic relationship between the reactions of wheat to *Puccinia graminis tritici* Eriks. and Henn., *P. glumarum tritici* Eriks., *Erysiphe graminis* DC., and pigmentation of the straw. The various relationships have been studied in 3 different wheat crosses, namely: H–44–24 × Marquis, Marquillo × H–44–24 and Garnet × Double Cross. The wheat material was studied in the sixth generation, and was unselected except that lines segregating for seedling reaction to *P. graminis* forms 21 and 36 were eliminated in F_4. The origin of the parental varieties, and the results of studies with *P. graminis* alone, have been reported in an earlier publication (10).

All the data on *Puccinia graminis* were obtained at Winnipeg, Canada, in the years 1929 and 1930. The rest of the work was done at Cambridge, England, in 1933–34.

The reactions to *Puccinia glumarum*[3] were determined by means of seedling tests made in the greenhouse. The tests with *P. graminis* were made in the field (mature-plant reactions) and in the greenhouse (seedling reactions). The mature-plant resistance of the variety H–44–24 to *P. graminis* is inherited independently of the seedling reaction. The two reactions are, therefore, considered separately in relation to the other characters.

The reactions to *Erysiphe graminis* were studied both in the greenhouse and in the field. The greenhouse reactions were determined by means of seedling tests. A heavy natural epidemic made an accurate field classification possible. The field notes were taken shortly after the wheat had come into head. There was no marked resistance to *E. graminis* in the cross Gar-

[3] The writer is indebted to Mrs. F. C. Bawden, School of Agriculture, Cambridge, for the cultures of *P. glumarum* used.

net × Double Cross so, naturally, no relationships could be established. The variety H–44–24 proved highly resistant both in the field and in the greenhouse. The lines of the crosses H–44–24 × Marquis and Marquillo × H–44–24 were, with few exceptions, definitely resistant, susceptible, or segregating. The seedling reactions determined in the greenhouse agreed very closely with the field reactions, so that the two will not be considered separately.

Under the conditions prevailing in the field at Cambridge in 1934, H–44–24 was characterized by a deep red coloration of the straw. In the H–44–24 × Marquis lines a classification according to the presence or absence of red pigment was very simple. The lines were definitely pigmented, nonpigmented, or segregating. In Marquillo × H–44–24 all the lines were more or less pigmented. In Garnet × Double Cross the distinction between pigmented and nonpigmented lines was fairly clear, though a few doubtful cases were encountered.

Seedling reactions to each of the two rusts were classified as resistant (R), moderately resistant (MR), semiresistant (SR), moderately susceptible (MS) and susceptible (S). In the case of the trials with *Puccinia graminis,* individual lines were marked R, MR, etc., according to the particular reaction. It was not considered necessary to record the reaction of individual seedlings, as most segregating lines had been previously discarded. The seedlings of each line were classified individually for their reactions to *P. glumarum,* and a mean value calculated for each line. For the purpose of calculating the means, the numerals 1 to 5 were substituted for the symbols R, MR, etc. The tests with *P. graminis* were not duplicated except in certain doubtful cases. The tests with *P. glumarum* were duplicated in the cross H–44–24 × Marquis, but not in the other two crosses.

The chief characteristics of the parental varieties are recorded in table 1. It is necessary to remember, in connection with the reactions to *P. graminis,* that "field reaction" refers to the reaction under field conditions of plants

TABLE 1.—*Description of the parental varieties in regard to the characters concerned*

Variety name	*Puccinia graminis* (field)	*Puccinia graminis* (greenhouse)	*Puccinia glumarum* (greenhouse)	*Erysiphe graminis*	Straw color
Marquis	S	S(forms 36 & 52)	MS	S	White
H–44–24	R	R(forms 36 & 52)	SR	R	Red
Marquillo	MR	MS(forms 36 & 52)	R	S	Red
Double Cross	MR	R(forms 21 & 35)	R	S	Pale red
Garnet	S	S(forms 21 & 35)	SR	S	Red

approaching maturity. Thirty or more physiologic forms were involved in the field epidemics. "Greenhouse reaction" refers to the reaction of seedlings under greenhouse conditions.

RESULTS WITH H–44–24 × MARQUIS

Puccinia glumarum and Field Reaction to *P. graminis*

The variety H–44–24 is extremely resistant to *Puccinia graminis* under field conditions at Winnipeg. Judged by the seedling reaction in the greenhouse, it varies from high resistance to moderate susceptibility, depending on the particular physiologic form used. The field, or mature-plant, resistance is inherited as a simple Mendelian dominant in the cross H–44–24 × Marquis and is independent of the seedling reaction (5, 10).

In this cross 114 lines were tested in duplicate with culture 41 (Botany School numbering) of *Puccinia glumarum*. Since it was not possible to maintain a constant temperature in the greenhouse, the two trials were conducted at different times. The first series was inoculated on the 5th of March, and the second on the 19th of April. Mean values of each line in each test were calculated. The mean values obtained in the duplicate trials were closely correlated, r being equal to .87. This indicates that the classification was fairly accurate. The distinction was, for the most part, between semi-resistance and moderate susceptibility; consequently, a certain amount of error in classification was inevitable.

In table 2 the wheat strains are arranged according to their field reaction to *Puccinia graminis* and seedling reaction to *P. glumarum*. The means of the *P. glumarum* reactions are based on the average value of the two trials. A close relationship between resistance to the two organisms is evident. It appears that the gene concerned in the difference between H–44–24 and Marquis in regard to their field reactions to *P. graminis* also plays the chief rôle in the inheritance of resistance to *P. glumarum*.

TABLE 2.—*Lines of the cross H–44–24 × Marquis arranged according to seedling reaction to Puccinia glumarum and field reaction to P. graminis. (P. glumarum means based on the average of duplicate trials)*

Puccinia graminis	*Puccinia glumarum*						
	[a]2.00	2.01–2.50	2.51–3.00	3.01–3.50	3.51–4.00	4.01–4.50	4.51–5.00
Resistant	1	6	14	5	5		
Susceptible				2	18	11	17
Segregating		2	3	9	12	6	2

[a] 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

TABLE 3.—*Lines of the cross H–44–24 × Marquis arranged according to seedling reaction to Puccinia glumarum and field reaction to P. graminis. (P. glumarum means based on the second trial)*

Puccinia graminis	*Puccinia glumarum*						
	[a]2.00	2.01–2.50	2.51–3.00	3.01–3.50	3.51–4.00	4.01–4.50	4.51–5.00
Resistant	4	8	10	6	3		
Susceptible					20	5	23
Segregating		1	4	5	17	3	5

[a] 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

An even closer relationship is evident if the mean values of the second trial to *Puccinia glumarum* are used. The explanation of this is probably to be found in the fact that the conditions in the greenhouse for the development of *P. glumarum* were better in April, when the second trial was made, than in March. The results are given in table 3.

It is clear from the data presented that field resistance to *Puccinia graminis* is very closely linked with the seedling reaction to *P. glumarum* in the cross H–44–24 × Marquis. Probably the same gene is responsible for the inheritance of resistance to the two organisms. It is, of course, possible that the relationship is due to the close linkage of two different pairs of genes. This possibility cannot be dismissed or confirmed without more extensive data.

The variability of the reactions to *Puccinia glumarum* within the resistant and susceptible classes of *P. graminis* is probably due, in part, to genetic causes. The evidence for this conclusion is derived from a study of the relationship between the duplicate trials with *P. glumarum* within the resistant and susceptible classes of *P. graminis*. If the variation in these two classes were random, there should be no correlation between duplicate trials. In the group resistant to *P. graminis* the correlation between duplicate trials with *P. glumarum* was $r = .46$ ($t = 2.64$). The three lines that gave mean reactions to *P. glumarum* of over 3.50 (see table 3) in the second trial were not included in the calculation of the correlation coefficient. They probably were wrongly classified as R to *P. graminis*. In the group susceptible to *P. graminis* the correlation between duplicate trials with *P. glumarum* was $r = .56$ ($t = 4.58$). It is evident, therefore, that there are one or more genes modifying the reaction to *P. glumarum* in the groups resistant and susceptible to *P. graminis*.

It appears from the above data that the inheritance of the seedling reaction to *Puccinia glumarum* form 41 and the field reaction to *P. graminis* is

mainly controlled either by one gene or by two closely linked genes. This result is significant in relation to certain wheat-breeding problems. It is remarkable that, although the field reaction to *Puccinia graminis* is not genetically associated with the seedling reaction to this organism (except in a very limited way, to be noted later), yet it is associated with the seedling reaction to *P. glumarum*. The most significant feature of this result is the provision of a method whereby selection for field resistance to *P. graminis*, in crosses involving H–44–24, can be made by a simple test in the greenhouse. The test is of particular value to breeders who cannot rely on an annual outbreak of stem rust in their experimental plots.

Puccinia graminis Form 36 and *Erysiphe graminis*

A somewhat intricate relationship between the reactions to *Erysiphe graminis* and form 36 of *Puccinia graminis* is evident in tables 4 to 7. In table 4 there is a deficiency of lines susceptible to both organisms; the deficiency, however, is not statistically significant. Consideration of only those lines characterized by mature-plant resistance to *P. graminis* (table 5) is very suggestive. The 5 lines susceptible to *E. graminis* are all resistant or moderately resistant to *P. graminis* form 36. So few lines are available for this comparison that the result cannot be considered conclusive. An interesting check on this result is afforded by a group of lines of the same cross selected for field resistance to *P. graminis*. Reactions to form 36 and *E. graminis* also were determined for these lines. The result is set forth in table 6. The agreement between the two independent results is very close,

TABLE 4.—*Distribution of H–44–24 × Marquis lines according to the reactions to Erysiphe graminis and form 36 of Puccinia graminis*

Erysiphe graminis	*P. graminis* form 36			
	R	MR	SR	S
Resistant	8	12	17	9
Susceptible	9	3	7	2

TABLE 5.—*Lines arranged according to the same characters as in table 4 but including only those lines possessing the field resistance of H–44–24 to Puccinia graminis*

Erysiphe graminis	*P. graminis* form 36			
	R	MR	SR	S
Resistant	4	2	3	2
Susceptible	2	3	...	...

and leaves little doubt regarding the reality of the relationship. The two sets of data are combined in table 7.

TABLE 6.—*Lines arranged according to the same characters as in table 5, but derived from a different set of H–44–24 × Marquis material, and all possessing the field resistance of H–44–24 to Puccinia graminis*

Erysiphe graminis	*P. graminis* form 36			
	R	MR	SR	S
Resistant	8	7	2	4
Susceptible	6	4	...	...

TABLE 7.—*Tables 5 and 6 combined*

Erysiphe graminis	*P. graminis* form 36			
	R	MR	SR	S
Resistant	12	9	5	6
Susceptible	8	7	...	...

The situation with regard to the three characters concerned is somewhat complex. We know from previous work (5) that there are two genes concerned in the inheritance of the seedling reaction to form 36. Since there are more than twice as many lines resistant to *Erysiphe graminis* as there are susceptible, in all probability more than one gene is involved in the inheritance of the reaction to this organism. Field resistance to *Puccinia graminis* in this cross is inherited as a simple Mendelian dominant. The relationship observed could be conditioned by the operation of four genes, as follows: R, a gene for field resistance to *P. graminis;* X, a gene for resistance to form 36; Y, a gene for resistance to form 36 and susceptibility to *E. graminis* which is expressed only in the presence of R; and Z, a gene for resistance to *E. graminis* which is epistatic to Y. The expected phenotypes on the basis of this theory are given in table 8.

The theory proposed is in accordance with the facts as far as the various combinations of characters are concerned. It explains why all lines resistant in the field to *Puccinia graminis* and susceptible to *Erysiphe graminis* are resistant to form 36. If this explanation be correct, the segregation for reaction to form 36 should be bifactorial in the field rust-resistant class, and monofactorial in the field rust-susceptible class (Y being nonfunctional in the latter). That the segregation is quite different in the two classes is indicated by the data in table 9.

TABLE 8.—*Expected phenotypes in the cross H–44–24 × Marquis in regard to the field reaction to Puccinia graminis, the seedling reaction to P. graminis form 36, and the reaction to Erysiphe graminis according to the theory proposed*[a]

Phentoype	Field reaction to *P. graminis*	Seedling reaction toform 36	Reaction to *E. graminis*
RXYZ	resistant	resistant	resistant
RXYz	"	"	susceptible
RXyZ	"	"	resistant
RxYZ	"	"	"
RXyz	"	"	"
RxYz	"	"	susceptible
RxyZ	"	susceptible	resistant
Rxyz	"	"	"
rXYZ	susceptible	resistant	"
rXYz	"	"	susceptible
rXyZ	"	"	resistant
rxYZ	"	susceptible	"
rXyz	"	resistant	susceptible
rxYz	"	susceptible	"
rxyZ	"	"	resistant
rxyz	"	"	susceptible

[a] Heterozygous lines are not considered. See also the text and tables 4 to 7.

TABLE 9.—*Lines of the cross H–44–24 × Marquis arranged according to the seedling reaction to Puccinia graminis form 36 and the field reaction to P. graminis*

Field reaction	*P. graminis* form 36			
	R	MR	SR	S
Resistant	17	3	8	4
Suspectible	9	13	14	10

Apart from the two relationships described, no linkages were evident between any two of the four characters concerned. The presence or absence of red pigment in the straw appeared to be independent of the reactions to the three diseases.

MARQUILLO × H–44–24

Parental Varieties

The variety Marquillo is moderately resistant to *Puccinia graminis* under field conditions. It is also resistant in the seedling stage, under greenhouse conditions, to certain physiologic forms of *P. graminis*. The inheritance of seedling and of mature-plant reactions is governed by the same genes in

crosses between Marquillo and susceptible varieties. In other words, Marquillo has no 'mature-plant' resistance in the sense that H–44–24 has (11). In the seedling stage, and probably under field conditions, Marquillo is highly resistant to *P. glumarum*. In view of these facts it is natural to expect the genetic behavior of resistance to the two rusts to be very different in this cross from that found in H–44–24 × Marquis.

Puccinia graminis (Field Reaction) and *P. glumarum*

In regard to the genetic relationship between resistance to *P. graminis* and *P. glumarum* some interesting facts have come to light. A total of 224 lines of this cross were used. One lot of 113 lines was inoculated with form 22 of *P. glumarum*, and the remainder with form 41. The two trials gave essentially identical results when considered in relation to the *P. graminis* reactions; consequently, only the data obtained with form 22 are given. Lines that segregated for reaction to *P. graminis* are not included in the tables. The *P. glumarum* tests were not duplicated.

TABLE 10.—*The lines of the cross Marquillo × H–44–24 arranged according to the seedling reaction to Puccinia glumarum and the field reaction to P. graminis*

P. graminis (field)	*P. glumarum* form 22				
	1.00[a]	1.01–2.00	2.01–3.00	3.01–4.00	4.01–5.00
Resistant	13	9	13	8	2
Semiresistant	3	2	.....	.....	4
Susceptible	9	4	.....	4	10

[a] 1.00 = R, 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

In table 10 the lines are distributed according to the field reaction to *Puccinia graminis* and greenhouse reaction to *P. glumarum*. As in the case of H–44–24 × Marquis, each seedling was classified for its reaction to *P. glumarum* and the mean calculated for each line. It is evident from the data in table 10 that the high resistance (1.00) to *P. glumarum*, characteristic of Marquillo, is not correlated with the field reaction to *P. graminis*. However, the semiresistance (3.00) of H–44–24 is definitely associated with field resistance to *P. graminis*. This is to be expected in view of the result obtained in H–44–24 × Marquis (table 2). It follows from this result that in Marquillo × H–44–24, as in H–44–24 × Marquis, selection for field resistance to *P. graminis* can be based on seedling tests in the greenhouse. Lines semiresistant to *P. glumarum* in the greenhouse will possess the high field resistance of H–44–24 to *P. graminis*. The results of previous studies (11) have

shown that the seedling reaction to form 21 of *P. graminis* may be used as a basis for selection for the field resistance of Marquillo.

Puccinia graminis Form 52 and *P. glumarum* Form 22

In the classification according to the seedling reaction to *P. graminis*, the reaction of individual seedlings was not recorded. The lines were classified as resistant, moderately resistant, etc., or segregating. Of the 113 lines classified for their reaction to *P. glumarum*, 11 lines were heterozygous for the *P. graminis* reaction, and the reactions of 6 were not determined. This leaves a total of 96 lines available for comparison. The relationship between the two reactions is illustrated in table 11. The lines fall into two well-

TABLE 11.—*The lines of the cross Marquillo × H–44–24 arranged according to the seedling reactions to Puccinia glumarum and P. graminis form 52*

P. graminis form 52	*P. glumarum* form 22				
	1.00[a]	1.01–2.00	2.01–3.00	3.01–4.00	4.01–5.00
Resistant	...	9	8	9	7
Moderately resistant	2	...	5	4	15
Semiresistant	17	3	.....	...	...
Susceptible	12	5	.....	...	...

[a] 1.00 = R, 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

defined groups; one more or less resistant to *P. graminis* and tending towards susceptibility to *P. glumarum*, and the other more or less susceptible to *P. graminis* and resistant or moderately resistant to *P. glumarum*. It is also possible that the difference between resistance and moderate resistance to *P. graminis* may be associated with the difference between moderate susceptibility and susceptibility to *P. glumarum*.

The actual number of genes concerned in the inheritance of reaction to these two rusts in this cross is not known. Since Marquillo is highly resistant, and H–44–24 semiresistant to *Puccinia glumarum*, and 22 of the 96 lines were definitely susceptible, it is certain that at least two genes are in operation. Judging from the variety of the types of infection by *P. graminis*, also, it is almost certain that two genes, at least, are involved. In view of these facts it is extremely improbable that the relationship observed can be explained on the basis of genetic linkage. It is probable that the genetic constitution that renders a plant resistant to *P. graminis* predisposes it to attack by *P. glumarum*. Likewise, plants genetically susceptible (more or less) to *P. graminis* are resistant or moderately resistant to *P. glumarum*.

In this cross there was no evidence of linkage of the other characters concerned, with the possible exception of *Erysiphe graminis* and the two rusts. There was a tendency for the lines susceptible to *E. graminis* to be concentrated in the MR and SR groups of *Puccinia graminis* form 52. However, the association was only just on the borderline of statistical significance.

GARNET × DOUBLE CROSS

Puccinia graminis Form 35 and *P. glumarum* Form 41

In this cross, resistance to *P. glumarum* is inherited independently of the field reaction to *P. graminis*. The reaction to *P. glumarum* is, however, closely associated with the greenhouse reaction to *P. graminis* form 35. The data illustrating this relationship are given in table 12. The susceptibility of Garnet to form 35 was not recovered in this cross, so that the *P. graminis* classification rested on the distinction between R and SR. The *P. glumarum* tests were made as in the other two crosses; but, as the majority of the lines were definitely resistant or susceptible, the remainder are grouped together in the table as SR and segregating. In all, 115 lines were tested; 24 were heterozygous for reaction to form 35, and these are excluded from the table. Neither of the tests was duplicated.

TABLE 12.—*The lines of the cross Garnet × Double Cross arranged according to their reactions to Puccinia graminis form 35 and P. glumarum form 41*

P. graminis form 35	*P. glumarum* form 41		
	R	SR & Seg.	S
Resistant	2	13	24
Semi-resistant	33	14	5

The relationship between the reactions to the two rusts is similar to that found in the cross Marquillo × H–44–24 (table 11); that is to say, resistance to one rust is closely associated with susceptibility to the other. The variety Double Cross (Marquillo × Marquis-Kanred) inherits its high resistance to *P. glumarum* from Marquillo. It seems, therefore, that the genes concerned in the resistance of Marquillo to *P. glumarum* are responsible for its susceptibility to certain physiologic forms of *P. graminis*. In table 12 there are two lines resistant to both rusts and five susceptible to both. This suggests that the relationship might be due to genetic linkage, and the lines giving similar reactions to both rusts would then represent the cross-over classes. However, as the susceptibility of Garnet to form 35 was not recovered (except in segregating lines) in spite of the fact that over 900 F_4 lines were tested, at least three genes, and probably more, must be in operation. The

reactions to *P. glumarum* likewise involve more than one gene, since Garnet is semiresistant and Double Cross highly resistant and 29 lines were definitely susceptible. In view of these facts it is unlikely that the relationship is due to genetic linkage. More probably it is due to the genes governing resistance to one rust being responsible for susceptibility to the other. If this be the case, the lines classified as resistant to both and those susceptible to both must be wrongly classified. It is unlikely that the errors were made in the *P. glumarum* classification, as the distinction between the R and S classes was quite clear. The distinction between the R and SR classes of *P. graminis,* however, was not always clear and errors were probably made. Unfortunately, form 35 of *P. graminis* has been lost, so that the result cannot be checked.

Puccinia graminis Form 21 and Straw Pigmentation

The variety Double Cross inherits immunity from *P. graminis* form 21 from Kanred. Crosses with other varieties show that it also possesses the Marquillo genes concerned in high resistance to form 21. The Kanred immunity is inherited independently of the Marquillo resistance (10).

TABLE 13.—*The lines of the cross Garnet × Double Cross arranged according to the pigmentation of the straw and reaction to Puccinia graminis form 21*

Straw color	*P. graminis* form 21			
	O[a]	R	SR	S
Red	25	4	6	12
White	25	4	14	1

[a] O = immune.

In table 13 the Garnet × Double Cross lines are arranged according to their reactions to form 21 and straw pigmentation. Of the 13 lines susceptible to form 21, only one has white straw. The reaction of this line to form 21 has been checked and its susceptibility confirmed. It is possible that an error in the classification of the straw color may have been made, and it also is possible that the line may be a result of natural crossing in F_2 with a white-straw variety. In any case there can be little doubt that the characters are genetically associated. The χ^2 test of independence gives a P value of less than .01. In the calculation of χ^2 the O and R classes were combined.

If the one white susceptible line is disregarded, the results can be explained by assuming that two genes are involved in the inheritance of straw color, and that the presence of one of them is essential to susceptibility to

form 21. According to this theory, purple lines may be either resistant or susceptible, while white lines are all more or less resistant.

Apart from the two cases of association described, the characters concerned appear to be inherited independently in the cross Garnet × Double Cross.

GENERAL DISCUSSION

The results of the investigation described in this paper are interesting in two respects, firstly; from the point of view of the production of disease-resistant varieties, and secondly; in regard to the nature of disease resistance in plants.

If reactions to all the important diseases of wheat were inherited independently, the development of varieties resistant to all would be merely a question of large-scale operation (that is, of course, assuming that no one organism is capable of attacking all varieties of the mutually interfertile species). By making appropriate crosses and studying a sufficiently large number of lines, it would be possible to combine in one variety resistance to all the fungous diseases concerned. It is evident from the results described in this paper that the reactions to different diseases may be correlated in inheritance. When resistance to two or more diseases is correlated, the breeding problem is simplified. An example of this is provided by the relationship between resistance to *Puccinia glumarum* and field resistance to *P. graminis* in the cross H–44–24 × Marquis. The direct association between resistance to one disease and susceptibility to another may render the combination of resistance to both in one variety impossible, at least for that particular cross. An example of such a relationship is provided by the results obtained with *P. graminis* form 52 and *P. glumarum* form 22 in the cross Marquillo × H–44–24. It is not necessary, however, to suppose that a relationship established in one cross will hold in another; in fact, it frequently does not. This is not surprising, since, in the case of *P. graminis*, for example, several sources of resistance, due to different genes, are available.

In regard to the fundamental nature of disease resistance, some of the results presented are of interest. If, as the data indicate, genes responsible for the inheritance of resistance to one disease may be concerned in susceptibility to another, any theory offered to explain the nature or cause of resistance to one must, at the same time, explain the nature or cause of susceptibility to the other. The cases of the relationship between resistance to *Puccinia graminis* and susceptibility to *P. glumarum* in H–44–24 × Marquillo and in Garnet × Double Cross are, in the writer's opinion, most easily explained by supposing that the internal condition of a plant that predisposes it to attack by *P. graminis* renders it resistant to *P. glumarum*. The possibility of the relationships described being due to genetic linkage cannot be entirely dismissed; but it is extremely improbable that genetic linkage will

explain the results, particularly when more than one pair of genes is concerned in the inheritance of the individual characters.

SUMMARY

In the cross H–44–24 × Marquis the seedling reaction to *Puccinia glumarum* was found to be closely associated with the mature-plant reaction to *P. graminis*. In the group of lines with mature-plant resistance to *P. graminis* all lines susceptible to *Erysiphe graminis* were resistant or moderately resistant to *P. graminis* form 36, and all lines susceptible or semiresistant to form 36 were resistant to *E. graminis*.

In Marquillo × H–44–24 semiresistance to *P. glumarum* was found to be associated with the mature-plant resistance of H–44–24 to *P. graminis*. Resistance in the seedling stage to form 52 of *P. graminis* was associated with susceptibility to *P. glumarum*, and, likewise, susceptibility to form 52 was associated with resistance to *P. glumarum*.

In Garnet × Double Cross the relationship between the seedling reactions to *P. graminis* form 35 and *P. glumarum* was similar to that found in Marquillo × H–44–24 in regard to *P. graminis* form 52 and *P. glumarum*. Susceptibility to *P. graminis* form 21 was associated with red pigmentation of the straw.

It is concluded that the relationships described are more probably due to a pleiotropic effect of the genes concerned than to genetic linkage.

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PERSISTENCE OF ERWINIA AMYLOVORA IN CERTAIN INSECTS

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Soon after the discovery of the cause of transmissible diseases of animals the medical profession was able to place a certain amount of responsibility in dissemination on insects, such as flies, fleas, cockroaches, bed bugs, etc. The feeding habits of many species of insects resulted in the spread of such diseases as typhoid fever and cholera (12). What was true for pathogens of animals became applicable to microorganisms causing diseases in plants. As early as 1891, Waite (14) directed the attention of plant pathologists to the fact that the fire-blight organism was spread by various insects: bees, flies, etc. Since that time a large number of diseases has been directly or indirectly connected with insects. However, experiments and observations were for the most part concerned with the external or mechanical transmission of diseases until quite recently, when some consideration has been given to the fate of microorganisms in the internal organs of insects.

Rand and Pierce (11), considering the internal transmission of pathogenic microorganisms, distinguish mechanical internal transmission in which the pathogens stay unchanged after passage through the alimentary tract without undergoing any appreciable multiplication or development within the insect body (*Bacillus pestis*), and biological internal transmission in which case the pathogens are able to multiply in insects and thus persist for a considerable time (*B. typhosus, B. tracheiphilus*). Both of these types of transmission have been shown for bacterial pathogens of plants and animals (1, 2, 8, 9, 10).

In order to obtain information as to the possible rôle played by some insects in biologic transmission of the fire-blight organism, the writers undertook an investigation under laboratory conditions employing *Drosophila melanogaster* (fruit fly), *Musca domestica* (house fly), *Lucilia seriata* (green bottle fly) and *Apis mellifica* (honey bee). Flies of these and other kinds are commonly found on the holdover cankers and in the blossoms of pear trees. Fruit-fly larvae and adults were found in decaying pear and apple fruits in Sonoma County, California, in January, 1935.

Drosophila flies were reared in sterile, cooked apple mash. The parent culture was procured from the Department of Genetics of this University. As soon as the larvae of *D. melanogaster* were plentiful they were transferred aseptically into another container, with apple medium contaminated with a 48-hour-old culture of *Erwinia amylovora* (Burrill) Winslow *et al.* Larvae were allowed to feed on the contaminated medium for 24 hours,

following which interval one lot of larvae was cultured in broth, while another lot was transferred into a fresh sterile apple medium for pupation. To culture the first lot, larvae were removed from the contaminated medium and sterilized with a 1 to 500 solution of $HgCl_2$ or 3 per cent Lysol solution for 3 minutes. After this the larvae were dropped into sterile nutrient beef broth of pH 6.9, crushed by means of a flamed flat needle and incubated at 28° C. Two kinds of control were included in this test. One consisted of inoculum from the flask of apple medium plus *E. amylovora,* in order to be certain of the viability of *E. amylovora,* and the other was surface-sterilized and washed, unerushed larvae that were allowed to remain in broth at 28° C. for 24 hours only. Any growth that appeared was plated out and diagnosed for *E. amylovora* with the pathogenicity test on fire-blight susceptible plants. The results of this first experiment were as follows: Out of 100 larvae 44 were positive for *E. amylovora.* The first control was positive for *E. amylovora,* while the second remained clear for 4 days, when it was discarded. Pupae, that developed from the larvae fed on contaminated medium for 24 hours and, subsequently sterilized with 1:500 solution of $HgCl_2$ for two minutes, were cultured after from 7 to 10 days. These were sterilized in 3 per cent Lysol, washed in 5 changes of sterile distilled water and crushed in tubes of a sterile nutrient broth. Of 65 pupae cultured after 7 days, 51 were positive for *E. amylovora.*

Fruit flies that developed from pupae were cultured as above 14 days after removal from the contaminated medium. Out of 78 flies, 56 were positive for *Erwinia amylovora.* Fifty empty puparia cultured revealed 40 with *E. amylovora.* Flies were left in the breeding medium to produce eggs, larvae, and pupae. Larvae of the second generation were found to carry organisms of *E. amylovora* in the internal organs. Pupae of this second generation were surface-sterilized and transferred into a freshly prepared apple medium. Out of 20 of these pupae cultured, 12 yielded a pathogenic form of *E. amylovora.* Newly emerged imagoes representing the flies of the second generation were now cultured. Among 36 of these flies, 19 contained *E. amylovora* in their viscera.

Thus it appears that the Drosophila flies may carry the organism from one generation to another when bred in the medium containing pathogenic organisms. This observation is in agreement with that of Leach (4, 5) and Johnson (3) who found that the bacterial flora of insects is to a large extent determined by the substrate on which it subsists. Due to the small size of the Drosophila eggs, it was not feasible to establish whether they carried the bacteria.

An experiment was performed to see how long the organisms of *Erwinia amylovora* are able to remain alive in the digestive organs of Drosophila

flies. Two hundred adult flies were fed on contaminated apple medium for 24 hours after which time they were transferred to another bottle that contained no food. The cotton plug of the bottle was wrapped with a blotting paper wetted with 1:500 $HgCl_2$ solution and a strip of the same kind of paper treated in the same way was allowed to extend about 2 inches into the test tube. This last procedure was undertaken in an attempt to disinfect the feet of the flies and thus reduce the chance of food contamination on their subsequent feeding. The flies were fed every 6–10 hours by removing the cotton plug and inserting a cork in the center of which was placed some apple medium. Methods of surface sterilization and culturing of flies of this experiment remained the same as those described earlier. The results are found in table 1. This table indicates that the fire-blight organisms may remain viable in the digestive organs of adult *Drosophila melanogaster* flies for as long as 6 days.

The faeces of Drosophila flies were likewise examined for *Erwinia amylovora* in the following manner. Before surface sterilizing, flies were introduced into a clean sterile test tube and allowed to defecate on the wall of the test tube. After this they were etherized and removed. Now, the faecal spots were touched with a sterile cotton swab moistened in sterile broth, dropped into broth which then was incubated at 28° C. Growth obtained was diagnosed for the fire-blight bacteria by inoculating shoots of *Pyrocantha angustifolia*. There was observed to exist a remarkable cor-

TABLE 1.—*Persistence of Erwinia amylovora in viscera of Drosophila melanogaster, Musca domestica, and Lucilia seriata*

Time interval		Hours					Days			
		0	17	24	48	72	4	5	6	9
Drosophila melanogaster	No. cultured	20	.....	15	20	22	25	18	20	20
	No. positive	20	.....	13	12	17	20	11	8	0
	Faeces	.....	.....	+	+	+	+	+	+	–
Musca domestica	No. cultured	10	.....	12	15	14	12	–	–	–
	No. positive	10	.....	12	10	8	0	–	–	–
	Faeces	.....	.....	+	+	+	–	–	–	–
Lucilia seriata	No. cultured	10	.....	10	13	18	18	11	–	25
	No. positive	10	.....	10	11	15	10	0	–	0
	Faeces	.....	.....	+	+	+	+	–	–	–

relation between the persistence of *E. amylovora* in viscera and the presence of it in defecated material (Table 1).

The common house-fly, *Musca domestica,* also was investigated in reference to the fire-blight organism. Sterilized horse dung to which a little commercial casein was added proved to be a satisfactory substratum.

In one experiment with *Musca domestica,* flies were allowed to feed on 10 per cent sucrose solution contaminated with *Erwinia amylovora* and then to deposit their eggs on the horse dung-casein mixture. These eggs were divided into two groups: one group was cultured in broth without any surface sterilization and another group was surface sterilized with 1:1000 solution of $HgCl_2$ for 2 minutes before culturing. Invariably eggs without sterilization carried *E. amylovora,* while sterilized eggs were always negative for this organism. Eggs deposited by *M. domestica* flies fed on contaminated sugar solution, produced larvae, pupae, and imagoes that harbored the pathogenic bacilli (*E. amylovora*).

In another experiment house flies were fed on sterile 10 per cent sucrose solution and allowed to deposit eggs in the horse dung-casein mixture. Larvae produced from these eggs were transferred to horse dung-casein mixture plus *Erwinia amylovora.* Forty-eight hours later 17 larvae were cultured after surface sterilization and were all found to harbor the bacilli of the fire-blight disease. Twelve pupae cultured from this lot were all contaminated internally with *E. amylovora.* Flies from similar pupae were cultured immediately after emergence in 2 different ways: first, they were surface sterilized with a 1:500 solution of $HgCl_2$ for 5 minutes and then crushed in sterile broth; second, they were thrust without sterilization into the nutrient broth, shaken for 3 minutes, and then discarded altogether. Thirty flies treated by the first method yielded 29 flies positive for *E. amylovora* and 24 flies treated by the second method contained 18 flies positive for *E. amylovora.* Forty empty puparia cultured in nutrient broth were 100 per cent positive for *E. amylovora.*

Seventy-five house-flies were fed on contaminated 10 per cent sugar solution for 24 hours and subsequently transferred into a new cage with food removed altogether but water supplied. These flies were cultured in much the same way as was Drosophila. Data on this experiment are included in table 1. It is evident that *Erwinia amylovora* remains in the digestive organs of the housefly for three days under laboratory conditions. Excrements of the housefly that had fed on contaminated food were found to contain viable organisms of *E. amylovora* soon after feeding and up to the fourth day thereafter.

To carry the investigation further green bottle flies, *Lucilia sericata,* were procured from the Department of Entomology of the University of California. Attempts to rear them in a manner similar to that used for *Musca*

domestica were unsuccessful. The experiment was therefore limited to adult flies. The flies were fed on contaminated 10 per cent sugar solution for 24 hours and subsequently transferred into a clean cage with sterile 10 per cent sucrose solution. Some flies were cultured immediately and some after various intervals of time. The results are found in table 1. Flies of *L. seriata* pass the organism of the fire blight through their alimentary canal uninjured and the faeces remain infective up to the fourth day, under laboratory conditions.

The honeybee, *Apis mellifica*, has been mentioned more often than any other insect in connection with the fire-blight disease. The writers aimed to study this insect in reference to the possible fate of the fire-blight organism when this organism is ingested by the bee. A large number of bees was collected in the field and confined to a cage. Honeybees do not adjust rapidly to confinement and a large number of them die soon after capture. Bees were fed on 10 per cent contaminated (*Erwinia amylovora*) sucrose solution for 24 hours and after that only on sterile sugar solution. This solution was changed every 2 hours to minimize the danger of recontamination with the organism. After 24 hours feeding, 5 bees were surface-sterilized in 5 per cent Lysol solution for 5 minutes, washed in 6 changes of sterile distilled water and cultured in broth by crushing with a sterile needle. All 5 yielded virulent cultures of the fire-blight organism. Now, the bees after surface sterilization were cultured in the following manner: the head and the thorax were pulled out with the flamed forceps while the abdomen was held with another pair of sterile forceps. In this way it was possible to separate the nectar stomach from the abdomen. The findings are tabulated in table 2. The data presented show that the fire-blight organism may remain viable in viscera and nectar stomachs of the honeybees for as long as 48 hours under controlled laboratory conditions.

TABLE 2.—*Persistence of Erwinia amylovora in nectar stomachs and other viscera*[a] *of the honeybee*

Time interval	Number of cultured:		Cultures positive for *E. amylovora*	
	Viscera	Nectar stomachs	Viscera	Nectar stomachs
24 hours after feeding	10	10	10	10
48 " " "	10	10	10	8
72 " " "	20	20	10	7
96 " " "	10	10	0	0
120 " " "	10	10	0	0

[a] By other viscera the writers mean abdomen when head, thorax, and nectar stomach are removed.

To ascertain whether *Erwinia amylovora* is able to stay alive in the mouth parts of the honeybee some 50 bees previously fed on contaminated sugar solution were cultured soon after feeding and 24 hours after feeding. In each case heads were surface-sterilized. No bacteria were isolated. Heads of another lot of 50 bees were cultured without any surface sterilization to learn how long the organism of *E. amylovora* may remain on it. These bees were fed on contaminated sugar solution and in addition sprayed with it to be sure of the presence of the organism on the heads. Ten bees cultured after each of the intervals indicated gave 10 positive when cultured immediately, 6 positive after 6 hours, 7 positive after 10 hours, 4 positive after 12 hours and all negative after 24 hours. This finding is in essential agreement with that reported recently by Pierstorff and Lamb (7). Attempts to isolate the organisms from faeces of bees were unsuccessful in a number of tests. The data show that the honeybees may ingest the pathogen with contaminated food, harbor it in their nectar stomachs and other viscera but destroy it, apparently by secretions in a relatively short time. These results offer a possible explanation of the lack of infection when known infested beehives are confined with blossoming trees (6, 7, 13).

From the point of view of epiphytology, the knowledge of transmission of disease by insects is of paramount importance, be it external or internal transmission. Obviously enough, the internal transmission and especially biologic internal transmission of a pathogen deserves especial consideration, since in this case the insect may retain the organism over a shorter or longer period unfavorable for development in the susceptible plant and thus the severity and duration of an epiphytotic may be greatly accentuated.

The experiments reported above seem to demonstrate that the dissemination of the fire-blight organism takes place by biologic transmission in addition to the known mechanical transmission.

SUMMARY

Larvae of *Drosophila melanogaster* and *Musca domestica* after feeding on a medium contaminated by *Erwinia amylovora* were found to contain the organism in their internal organs. The bacteria persisted through the pupa to the adult in both of these insects.

Eggs of *Musca domestica* from contaminated females carried the organism externally but not internally.

Adults of *Drosophila melanogaster*, *Musca domestica* and *Lucilia seriata* harbored the organism internally for 6, 3, and 4 days, respectively.

The bacteria remained viable in the viscera of honeybees for 48 hours but were not recovered from the heads after 12 hours from the time of contamination.

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RESISTANCE TO CLADOSPORIUM FULVUM[1]

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The study of resistance in the genus Lycopersicum to *Cladosporium fulvum* Cke. and the development of tomatoes resistant to leaf mold is now receiving recognition in several countries. An account of the progress made and the possibilities that the work so far indicates may be of interest to plant pathologists at this time. That the control of this disease, which has wrought havoc to the greenhouse tomato industry, may be accomplished through the science of genetics now seems probable.

Cladosporium fulvum is narrowly confined to the tomato, *Lycopersicum esculentum* Mill. (20), (35), (15, 17), (7, 8), (31). The cherry and plum tomatoes (f. *cerasiforme* Alef. and f. *pruniforme* Alef.) are susceptible, but the red currant tomato, *L. pimpinellifolium* Mill. is completely resistant (31), (17), (7, 8). In the writer's studies the potato-leaved (f. *grandifolium* Bailey), as represented by the variety Wayahead, the pear (.f *pyriforme* Alef.), and the plum tomatoes were very susceptible, and yellow cherry was more resistant than red cherry, although both were quite susceptible. In the closely related genera Physalis and Solanum, all the members tested proved resistant, but the writer's attempts to hybridize members of these genera with the cultivated tomato failed.

Certain varieties of the cultivated tomato (f. *vulgare* Bailey) have been reported to possess some resistance to leaf mold. This resistance is expressed by less growth of mycelium and less sporulation than occur on susceptible varieties and by the production of yellow blotches centering around the point of infection with but a scant growth of fungus. The foliage is slower to become infected, at least the incubation period appears to be longer, and the vines remain green longer than those of susceptible varieties, although under continued favorable conditions for infection, which are frequent in the fall greenhouse cropping season, the foliage of these somewhat resistant varieties ultimately becomes badly diseased. Therefore, under commercial growing conditions, the disease may affect these varieties seriously enough to be a limiting factor in the production of fruit.

Reported observations regarding the resistance of varieties of the common cultivated tomato to leaf mold are not consistent (Table 1). This appears to be due to variability in the growing conditions, where small differences influence the degree of infection, rather than to the existence of different strains of the fungus. In the sense of possessing the greatest

[1] Contribution No. 216 of the Massachusetts Agricultural Experiment Station.

TABLE 1.—*Varieties of tomato reported most resistant to Cladosporium fulvum*

Variety	Alexander (7)	Bewley (9)	Boock (10)	Guba (15), (16)	Heydemann (21)	Jagger (22)	Makemson (23)	Newhall (24)	Norton (25)	Quinn (27)	Rath (29)	Sengbusch and (31) Loschakowa-Hasenbusch	Small (33, 34)	Springer (36)
Frogmore Selected				−						−			+	
Lucullus	−		+	−							+	−		
Maincrop	+			+				+					+	
Norduke	+			+		+							+	
Satisfaction	+			−				+					+	
Sterling Castle	+	+	+	−		+			+	−		+	+	
Stone	−			−			−		+			−		
Tuckswood	−		−	−	+						+	−		+
Up-to-Date	+	+		+		+						−	+	
Riverside × Up-to-Date (Bewley)				+										
Stonnor's M.P. × Up-to-Date (Bewley)				+										
Maincrop × Up-to-Date (Bewley)				+										
E S 1 × Up-to-Date (Bewley)				+										
Norton	−			+										

+ Indicates resistant
− Indicates not resistant

measure of resistance and of showing value for developing hybrids somewhat resistant to leaf mold, the varieties Maincrop, Norduke, Up-to-Date, Norton, and certain hybrids originated by Bewley[2], appeared promising in the writer's studies, while Frogmore Selected, Lucullus, Stone, and Tuckswood, which have been reported to show resistance, proved very susceptible. Sterling Castle and Satisfaction were somewhat resistant, but also too susceptible to be considered of positive value in hybridization work.

Some reports of progress have appeared relating to the hybridization of tomatoes for resistance to leaf mold (9), (24), (16, 17), (31), (4, 5, 7). In these investigations, the varieties Maincrop, Up-to-Date, Sterling Castle, Satisfaction, and Norduke have rather consistently been found to offer most promise as a source of resistance for hybridization purposes (Table 2). The resistance of these varieties, as is shown in crosses of Sterling Castle, rests on recessive factors (31), (7). Since the progeny of these crosses

[2] In 1931 the writer received from Dr. W. F. Bewley, Director of the Experimental and Research Station, Cheshunt, England, seed of F4 selections of hybrids of Up-to-Date with Riverside, Stonnor's M.P., Maincrop, and E S 1.

TABLE 2.—*Crosses of standard varieties of tomato developed for resistance to Cladosporium leaf mold.*

Alexander (4, 5, 7)
- Sterling Castle × Marhio
- Sterling Castle × Globe
- Satisfaction × Marhio
- Satisfaction × Globe

Bewley (9)
- Up-to-Date × E S 1
- Sterling Castle × E S 1
- Up-to-Date × Riverside
- Up-to-Date × Tuckswood
- Up-to-Date × Maincrop
- Up-to-Date × Manx Marvel
- Up-to-Date × Stonnor's M.P.

Newhall (24)
- Satisfaction × Bonny Best
- Satisfaction × Marhio
- Maincrop × Bonny Best
- Maincrop × Marhio

Sengbusch and Loschakowa-Hasenbusch (31)
- Sterling Castle × Susceptible varieties

Guba (16, 17, 18)
- Up-to-Date × Norduke
- (Up-to-Date × Riverside) × Norduke
- (Up-to-Date × Stonnor's M.P.) × Norduke
- (Up-to-Date × Maincrop) × Norduke
- (Up-to-Date × E S 1) × Norduke
- Maincrop × Norduke

have seriously succumbed to infection under conditions favoring epidemics of leaf mold, more recent reports show that these efforts are being abandoned (7), (18). In the work of the writer even the reaction of the progeny of crosses of the somewhat resistant English types, such as Maincrop, Up-to-Date, and the Bewley hybrids with the somewhat resistant large-fruited American variety Norduke, was disappointing under the usual greenhouse conditions favorable for the disease.

Efforts to develop a desirable greenhouse tomato resistant to leaf mold have been confined largely and more recently to the hybridization of *L. pimpinellifolium* with *L. esculentum* f. *vulgare* (31), (17, 18), (6, 7, 8), and Wenholz.[3,4] It appears fortunate that this work is being done in different parts of the world, for it is believed that the objective of developing desirable greenhouse types of tomatoes for each particular market will be more readily achieved by the efforts of several investigators. Immunity has been shown to be expressed by a single dominant Mendelian factor. Excellent studies of other inheritance characters resulting from the hybridization of red currant with standard tomatoes have been reported by Groth (11, 12, 13, 14), Price and Drinkard (26), and others.

[3] In a letter to the writer dated May 13, 1935, it was learned that Dr. H. Wenholz of the Department of Agriculture, New South Wales, has also engaged in the hybridization of red currant with standard tomatoes for developing resistance to fusarium wilt. Some lines of his many crosses carried as far as the 6th generation for size of fruit and resistance to wilt were sent to the writer for study of their reaction to cladosporium leaf mold. In this study none showed resistance to cladosporium.

[4] The extensive work of Agerberg *et al.* (1, 2, 3) and Schmidt (30) to explain the high resistance of *L. pimpinellifolium* Mill. to *Cladosporium fulvum* Cke. unfortunately is referable to *Trichothecium roseum* (Pers.) Lk., a saprophyte (Raabe and Sengbusch (28)). It is hoped that further work at Müncheberg, Germany, will correct the disappointment that has resulted from this error.

It now appears definitely probable that large-fruit tomatoes, completely resistant to the disease, will be developed, which should be of great value to the greenhouse-tomato industry. With the purpose solely of developing an early-fruiting tomato for North Dakota, Yeager (37) hybridized the red currant with Bison and subsequently crossed selections of this hybrid of determinate growth habit with Pink Heart for size. The manifestation of earliness and unusual flavor offered by crosses of the red currant with common standard varieties (32), (19) would seem to have far reaching value aside from the matter of resistance to cladosporium leaf mold if the inference is true that such tomatoes may serve in the human diet as substitutes for orchard fruits.

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PHYTOPATHOLOGICAL NOTES

A Method for the Isolation of Actinomycetes from Scab Lesions on Potato Tubers and Beet Roots.—Many taxonomists consider the Actinomycetaceae and Mycobacteriaceae as families of the order Actinomycetales. A mixture of sodium hypochlorite and sodium hydroxide is used in the isolation of certain members of the second family.[1] It was suggested by W. H. Burkholder that such a mixture might be of value in the isolation of plant pathogenic actinomycetes. Since calcium hypochlorite is a common laboratory disinfectant this was substituted for the sodium salt.

Preliminary trials were conducted and the following technique was found satisfactory. Calcium hypochlorite is prepared as described by Wilson.[2] Ten grams of fresh calcium hypochlorite is thoroughly shaken in 140 cc. of tap water. This is allowed to stand a few minutes and then filtered. Immediately before using, 1 part of sodium hydroxide solution (250 grams of sodium hydroxide dissolved in water and made up to 1000 cc.) is added to 3 parts of the above filtrate.

Tubers are placed in the above mixture for 2 minutes, removed, and, without washing, a slice is cut to remove a lesion and the underlying healthy tissue, using a sterilized scalpel. This slice is placed in a few drops of sterilized distilled water in a flamed mortar and thoroughly triturated with a flamed pestle. After triturating the tissue additional water is added to the mortar and the diluted suspension transferred to a sterilized tube by pipetting. The use of a tube of sterilized water with a pipette inserted through the plug greatly facilitates this procedure.

After a number of tubes of the suspension have been prepared 1 or 2 drops from a tube are placed in the bottom of a sterilized Petri dish. About 15 cc. of Waksman's[3] egg-albumin agar,[4] which has been melted and cooled to 45° C., is then poured into the dish. The dish is carefully rotated to distribute the suspension through the medium. Colonies develop in from 2 days to 3 weeks, depending on the strain present and the incubation temperature.

[1] Park, W. H., A. W. Williams and C. Krumwiede. The bacillus and bacteriology of tuberculosis. p. 492–526. *In* Pathogenic Microorganisms. 9th ed. 819 pp. Lea and Febriger, Philadelphia. 1929.

[2] Wilson, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. 2: 420–427. 1915.

[3] Waksman, S. A. Cultural studies of species of Actinomyces. Soil Sci. 8: 71–207. 1919.

[4] "Egg-albumin agar. Dextrose, 10 gm.; K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; $Fe_2(SO_4)_3$, trace; egg albumin, 0.15 gm.; agar, 15 gm.; distilled water, 1000 cc. The egg albumin is dissolved in N/10 NaOH until neutral to phenolphthalein, then added to the warm medium."

The writer has used this method since the spring of 1930 with comparatively little trouble from contamination by other microorganisms. This disinfestation method suppresses bacterial contaminants. When certain other media, as potato-dextrose or beef-extract agar, are used instead of Waksman's agar, fungal contaminants may overrun the dish before the Actinomyces colonies develop sufficiently for transferring. No difficulty has been experienced in isolating Actinomyces from scabbed tubers at any time after the lesions become evident.—C. F. TAYLOR, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Physalis subglabrata: A Natural Host of Bacterium angulatum.[1]—All attempts to control the two bacterial diseases of tobacco, angular leaf spot and wild fire, based on the assumption that tobacco is the only host, have failed. In spite of all precautions to prevent infection from previous tobacco crops, leaves protruding through a tear in the cotton covering of the bed may develop a few wild-fire or angular leaf spots with no evidence whatever of any other infection. Closely boxed beds are usually freer from infection than nonboxed beds. Perfectly clean plants may be set in the field only to develop one or the other or both diseases following a rainy period.

The writers have believed that infection was carried over winter in weeds, but have previously failed to obtain evidence, other than observational, in favor of this hypothesis. Inoculation studies of J. Johnson *et al.*[2] favored the weed-host theory. They claimed a wide host range for *B. tabacum*. Clayton[3] showed, however, that the bacterial toxin present in broth used as inoculum was responsible, in the species studied, for the development of wild-fire spots. He was unable to demonstrate extensive multiplication of the organism except in species of Nicotiana, and concluded that only species of Nicotiana should be regarded as hosts of *Bacterium tabacum*. Beach[4] observed ground cherry plants (*Physalis virginiana*) with typical wild-fire lesions, growing near affected tobacco. The spots centered on injuries made by flea beetles. He isolated *B. tabacum* from these spots and believed that he had evidence that the parasite had multiplied within the leaf. He concluded, however, that "the results obtained with *Bacterium tabacum* upon *Physalis virginiana* seem to correspond in most respects to those reported by Clayton for a series of garden plants not belonging to the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Johnson, J., C. M. Slagg and H. F. Murwin. Host plants of *Bacterium tabacum*. Phytopath. 14: 175–180. 1924.

[3] Clayton, E. E. Toxin produced by *Bacterium tabacum* and its relation to host range. Jour. Agr. Research [U. S.] 48: 411–426. 1934.

[4] Beach, W. S. Control of tobacco wildfire (second report). Penn. Agr. Expt. Sta. Bul. 322, 1935.

genus *Nicotiana.*" After reading Beach's report, the present writers concluded, on the contrary, that the key to the solution of the sudden appearance of wild fire had probably been found. Consequently, plants of smooth ground cherry *Physalis subglabrata* M. & B. growing in pastures and fencerows at a distance from tobacco on the Experiment Station farm were immediately examined and found to be extensively spotted. Bits of tissue from the spots, examined microscopically, were found to be teeming with bacteria having motility typical of a polar flagellate organism. Bits of the spots dropped into broth used as inoculum on tobacco plants produced typical angular leaf spot. From 37 of 39 plants of *Physalis subglabrata* and from the only plant of clammy ground cherry worked with, typical angular leaf spot was obtained on tobacco plants, from which pure cultures of *B. angulatum* were obtained at will. A single colony from a typical angular leaf spot on tobacco was found to contain two strains of *B. tabacum,* a smooth, and a somewhat rough concentric colony strain, each of which caused typical wild-fire spots on tobacco.

The first collections were made October 31, two months after tobacco had been cut. Most of the inoculation studies were made after the groundcherry tissue had dried. As the weeds were growing at a distance from tobacco plants there was no opportunity for them to have become infected by drip from diseased tobacco plants. If the bacteria originated in tobacco, they must have been carried in relatively small numbers to the groundcherry where they became established and multiplied. The affected groundcherry leaves had all been gnawed by flea beetles. Beach believed that flea beetles were unable to transmit wild fire, although Wolf[5] had demonstrated in 1922 that they were able to do so. The writers found in the spring of 1935 that *Bacterium angulatum* could be isolated from the bodies of flea beetles and garden flea hoppers. Flea beetles feed upon physalis in the spring of the year and later migrate to tobacco beds, and in large numbers to fields recently set with tobacco. Injury to recently set plants is often extensive. *Bacterium tabacum* and *B. angulatum* both live through the winter in tobacco trash left out-of-doors, so there is no reason for believing that they will not live in affected physalis leaves also. Reinfection of Physalis could readily occur in the spring, and migration of flea beetles from it to tobacco could account for a part, at least, of plant-bed and field infection.

The evidence obtained up to the present seems sufficient to prove that *Physalis subglabrata* is a natural host of *Bacterium angulatum* and that plants of this species growing at some distance from tobacco go into the winter heavily infected. Sudden outbreaks, and equally sudden subsidence, of wild fire in a region may depend upon the extent to which Physalis becomes infected in the fall. Future work on the epiphytology of angular leaf spot and wild fire must take *Physalis subglabrata* and perhaps other

[5] Wolf, F. A. Wildfire of tobacco. N. C. Agr. Expt. Sta. Bul. 246. 1922.

solanaceous weeds into consideration.—W. D. VALLEAU AND E. M. JOHNSON, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

FIG. 1. Fasciated leader of European birch. A indicates position of twigs shown in figure 2. Approx. × 0.4.

Fasciation of Betula pendula dalecarlica.—On October 3, 1935, a striking case of fasciation was found on the tip of the main stem of a 25-foot

European birch, *Betula pendula dalecarlica* Schneid., in Washington, D. C. The rest of the tree appeared to be perfectly normal.

FIG. 2. Non-separated buds and twigs of fasciated European birch at end of branch A of figure 1. Approx. × 1.

The flattened and twisted development of the leader is shown in figure 1. Near its extreme tip (Fig. 2) is a cluster of buds that failed to separate. The contour of the twigs and their partial separation below the tip clearly indicate that the flat band-like expansion is at least partly caused by the failure of the twigs to separate and is not the abnormal development of a single branch.

Although fasciation is very common on some plants, in the literature there are comparatively few references to it on deciduous trees. The failure to find any reference to fasciation on birch leads one to conclude that the disease is rare and of no economic importance.—MARVIN E. FOWLER, Division

of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

Powdery Mildew of Potato in New Jersey.—During February, 1935, a leaf-spot disease appeared on some potato plants of the variety Green Mountain growing in the greenhouse at The Rockefeller Institute for Medical Research, Princeton, New Jersey. The spots began as small, irregularly shaped areas not more than 2 mm. in diameter. They were most numerous on the upper surfaces of leaves that were nearing maturity. As the spots enlarged, their color darkened. Confluent spots sometimes involved large areas of tissue. The disease persisted on potted plants in greenhouses during the past summer, but was not observed in nearby potato fields.

In periods of cloudy weather the fluffy white mycelium of a fungus developed over affected areas (Fig. 1). The fungus appeared in spots on both the upper and lower surfaces of leaves, on leaf petioles, and on stems. Microscopic examination of fresh leaf tissues and of stained sections of leaves showed it to be a powdery mildew. Numerous barrel-shape conidia borne in chains on short conidiophores were observed. When germinated in water, the conidia produced short, straight germ tubes. The mycelium was found to be superficial and attached to epidermal cells by means of globoid haustoria. As no perithecia were produced, it has not been possible to determine the species to which the fungus belongs, but it is presumed to be *Erysiphe solani* mentioned by Vaňha in 1902.[1] The conidia were found to have an average length of 34.19 ± 3.08 μ and an average width of 18.09 ± 1.61 μ, as determined by measurements on 100 mature spores taken at random from leaf spots. These average dimensions are in fairly close agreement with those reported by Müller[2] for the conidia of a mildew occurring on potatoes in Germany. Potato mildew has been recorded from France,[3, 4] Russia,[5] and Peru,[6] but, so far as the writer is aware, it has not been reported previ-

[1] Vaňha, J. Eine neue Blattkrankheit der Rübe. Ztschr. Zuckerindus. Böhmen 27: 180–187. 1902–03.

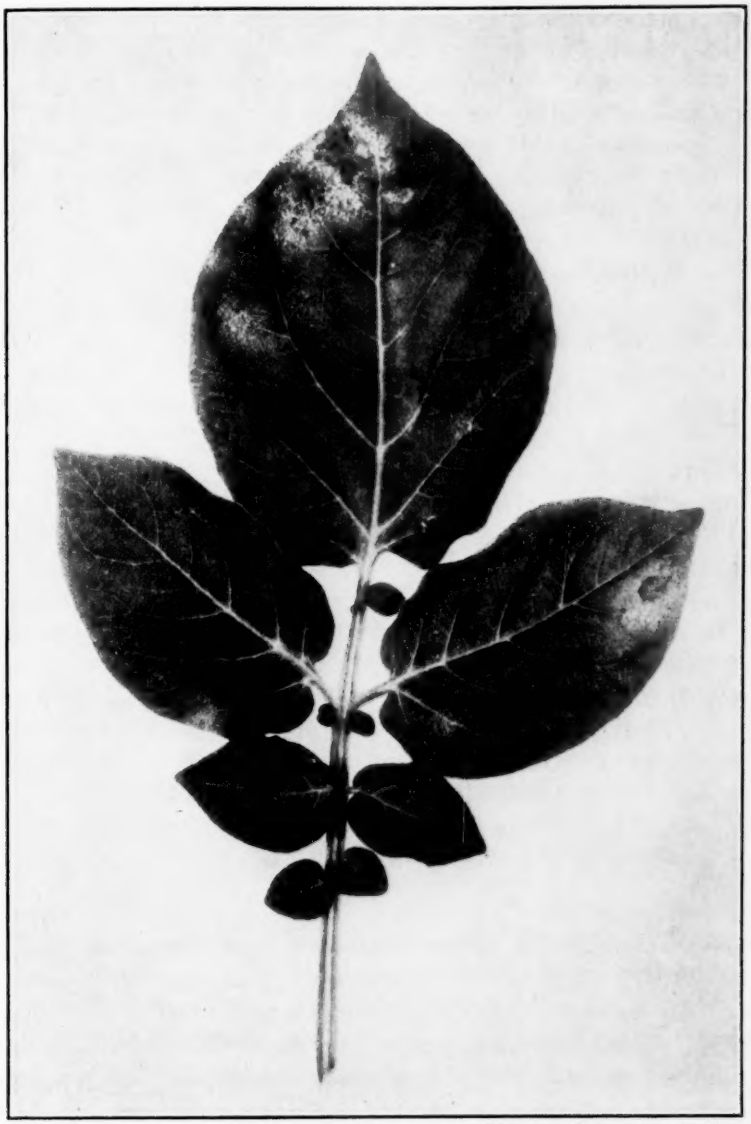
[2] Müller, K. O. Ueber den "echten Mehltau" der Kartoffel. Nachrichtenbl. Deutsch. Pflanzenschutzd. 8: 19–20. 1928.

[3] Ducomet, V. Oïdium de la pomme de terre et Oïdium de la betterave. Soc. Path. Vég. France Bull. 8: 153–154. 1921.

[4] Marchal, P., and E. Foëx. Rapport phytopathologique pour les années 1926–1927. Ann. des Épiphyties [France] 13: 383–454. 1927.

[5] Jaczewski, A. A. Parniatnaia Knizhka ialia zapiseĭ dannykh fitopatologichkogo osmotra Kartofelnykhs nasazhdeniĭ. [Notebook for entering data obtained from the phytopathological survey of potato crops.] A. A. Jaczewski Mycological Laboratory, Leningrad. 1926. Review in Rev. Appl. Mycol. 6: 250. 1927. In Russian. (Original article not seen.)

[6] Abbott, E. V. Further notes on plant diseases in Peru. Phytopath. 21: 1061–1071. 1931.



Photograph by J. A. Carlile

FIG. 1. Potato leaf affected by powdery mildew.

ously from the United States.—L. O. KUNKEL, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Elsinoe fawcetti, the Perfect Stage of the Citrus Scab Fungus.—A myriangiaceous ascomycete, typical of the genus Elsinoe and interpreted as

the perfect stage of the citrus-scab organism, was found recently on scab lesions on ripe rind of Satsuma orange, *Citrus nobilis unshiu* Sw. (Fig. 1-A). The specimen, consisting also of scabbed green rind and leaves of Satsuma, was collected on May 28, 1935, at M'Boy, São Paulo, by A. O. Martins, in connection with routine inspection of citrus in this locality, and sent to the Phytopathological Section of the Instituto Biologico de São Paulo. It was retained particularly for the purpose of obtaining a culture record of *Sphaceloma fawcetti* from this variety of orange. The isolation (No. 228C, Phytop. Sect. Inst. Biol. de São Paulo) was made on May 31, the day the specimen was received, by means of platings from rind lesions.

Dark punctiform fungus masses on the lesions (Fig. 1-B and C) were sectioned on Aug. 21, 1935, and these proved to be the ascomata of an Elsinoe (Fig. 1-D to I). An attempt was made to establish the relationship of the newly found fungus with *Sphaceloma fawcetti* by means of cultures from ascospores (Fig. 1-J); but, unfortunately, they produced no growth. Nevertheless, it is believed that this ascomycete is unquestionably the perfect stage of *S. fawcetti*. In the first place, the specimen is typical of citrus scab and the conidial stage of *S. fawcetti* is present on the younger lesions of the specimen, *i.e.*, the leaf lesions. Moreover, the culture isolated from scrapings of the rind lesions is typical of this fungus. Secondly, the occurrence of the Elsinoe on the scabs and its close resemblance to other species of the same genus, for example, *E. ampelina* (de By.) Shear, *E. piri* (Wor.) Jenkins, *E. phaseoli* Jenkins, that have been shown to be the perfect stage of other species of Sphaceloma, is sufficient indication of the genetic relationship between the newly discovered ascomycete and the citrus-scab organism. Since the Elsinoe has not been described heretofore, the name *Elsinoe fawcetti* is proposed.

Elsinoe fawcetti n. sp.

Ascomata more or less scattered, pulvinate, dark brown, circular to elliptical, 38–106 × 36–80 μ; epithecium composed of dark colored pseudoparenchyma, 5–9 μ thick; asci 1 to 20 or more in a single ascoma, distributed in the lighter colored stromatic region beneath the epithecium, globose to ovoid, 12–16 μ diameter, wall of unexpanded ascus thickened in upper portion; ascospores hyaline, oblong-elliptical, 10–12 × 5–6 μ; 2–4 celled, usually constricted at the middle septum, upper half of spore thicker and shorter, lower half thinner and longer; epispore 1.2 μ. Conidial stage, *Sphaceloma fawcetti* n. sp. (p. 101).[1]

Ascomata plus minusve sparsa, pulvinata, atro-brunnea, orbicularia vel elliptica, 38–106 × 36–80 μ; epithecium obscuro-pseudoparenchymaticum, 5–9 μ crassum; asci 1–20 vel plures in ascomate singulo, sub epithecio in

[1] Jenkins, A. E. The citrus scab fungus. Phytopath. 15: 99–104. 1925.

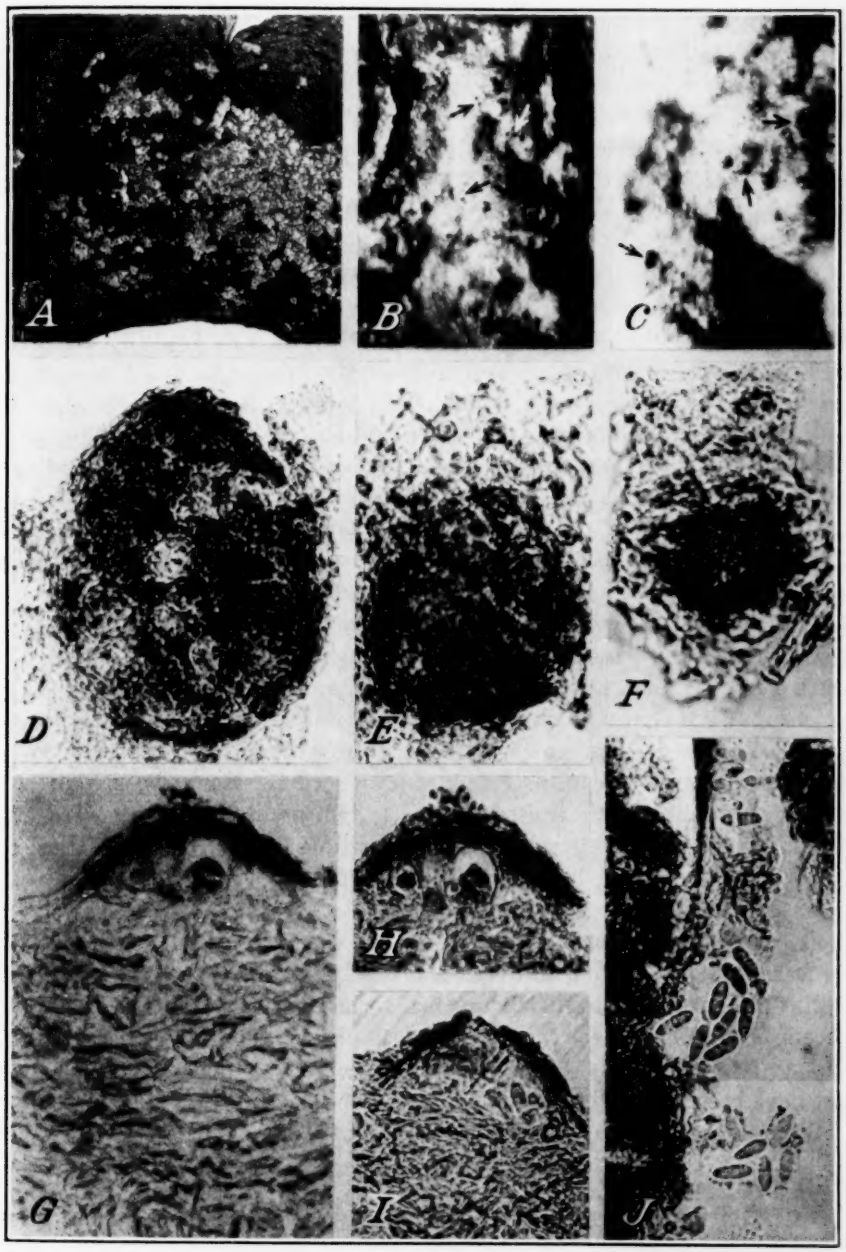


FIG. 1. *Elsinoe fawcetti* on rind lesions of Satsuma. A. Part of type (×1). B. and C. Ascomata on scabs (B ×22; C ×60). D–E. Ascomata; circular outline of asci indistinctly visible through epithecium. F. Ascoma with single ascus. G–I. Sections of scab with ascoma. H. Same as G, but stained with cotton blue. J. Ascospores. (D–J ×500).

regione stromatica pallidiore dispositi, globosi vel ovoidei, 12–16 μ in diam.; asci inexpansi superne crasse tunicati; ascospori hyalini, oblongo-elliptici, 10–12 × 5–6 μ, 2–4-cellulati, plerumque ad septum medium constricti, parte superiore crassiore et breviore, parte inferiore tenuiore et longiore; episporium 1.2 μ crassum.

On *Citrus nobilis unshiu* Sw., Brazil, São Paulo (Bairro de M'Boy) Chacara de Sumakiti Morita, May 28, 1935, A. O. Martins. In Herb. Phytopath., Instituto Biologico de São Paulo, Brasil, No. 2041 (Type of perfect stage). Part of specimen in Mycol. Coll. Bur. Plant Industry, U. S. Dept. Agric., Washington, D. C.—A. A. BITANCOURT, Instituto Biologico de São Paulo, Brazil, and ANNA E. JENKINS, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Reaction of the Victoria Oat Variety to Crown Rust.—The high resistance of the Victoria oat variety (C. I.[1] No. 2401) to crown rust (*Puccinia coronata avenae* Eriks. and Henn.) in North America was first reported by Murphy and Stanton.[2] This unusual resistance to crown rust, as well as high resistance to loose smut (*Ustilago avenae* (Pers.) Jens.), and covered smut (*U. levis* (Kell. and Sw.) Magn.), has resulted in the rather wide use of Victoria by oat breeders as a source of resistance to these diseases.

Unfortunately, some of the Victoria stocks do not react homozygously to crown rust. Numerous pure-line selections have been obtained from the variety and tested with different physiologic forms of the rust. While all of these lines were apparently morphologically identical, a few were completely susceptible to various forms of crown rust. However, a high percentage of the lines were resistant to all the forms to which they were subjected. The presence of one of these susceptible lines already has been reported by the writer,[3] but its practical significance was not emphasized. There is evidence that Victoria embraces also some smut-susceptible lines. Their presence in the original mass stock of Victoria has negated the value of several early and important crosses made by the writer and his coworkers in that these susceptible lines happened to be used as parents. Therefore, it seems desirable to report the fact and emphasize the importance of using as parent material only those pure-line selections of the original Victoria that have been definitely tested for their reactions to crown rust and smut.

Stanton and Murphy[4] previously have stated the lot of seed accessioned

[1] C. I. refers to accession number of the U. S. Dept. Agr., Bur. Plant Indus., Division of Cereal Crops and Diseases.

[2] Murphy, H. C., and T. R. Stanton. Oat varieties highly resistant to crown rust. Jour. Amer. Soc. Agron. 22: 573–574. 1930.

[3] Murphy, H. C. Effect of crown rust infection on yield and water requirement of oats. Jour. Agr. Research [U. S.] 50: 387–411. 1935.

[4] Stanton, T. R., and H. C. Murphy. Oat varieties highly resistant to crown rust and their probable agronomic value. Jour. Amer. Soc. Agron. 25: 674–683. 1933.

under C. I. No. 2401 represents an artificial population of the selections 64q, 64r, and 64t. These original selections were tested by the writer and found not to be pure within themselves in so far as concerned their reaction to forms 1, 3, 7, 16, 30, and 33 of crown rust. Pure lines were obtained from each of these original selections, which were completely susceptible to one or more of the above-mentioned forms. Although like Victoria, nearly all these lines were resistant to all forms used, the original selections each contained a larger proportion of susceptible types than are found in Victoria. Apparently, Victoria has been purified since these original selections were massed, or these original selections have been subjected to more mixture than has the Victoria variety.

Except for the presence of these lines in Victoria that are susceptible to crown rust and possibly to smut, this variety has been resistant to 37 physiologic forms of crown rust collected in the United States, Mexico, and Canada, in the 9-year period from 1927 to 1935 and to all collections of smut used in field and greenhouse studies at Ames, Iowa, during the same period. Victoria does not show so high a type of resistance to certain forms of crown rust as does Bond (C. I. no. 2733). When grown immediately adjoining heavily rusted plots, its yield may be reduced because of severe leaf necrosis resulting from primary infection. Under field conditions, however, where the amount of infection is naturally restricted because of the partial or complete elimination of the source for secondary and subsequent infections, a strain of oats endowed with Victoria's resistance to crown rust has ample protection, even when heavy epiphytotics are present. Although Victoria is poorly adapted to the principal oat-growing regions of the United States and is further undesirable because of the presence of a heavy, twisted, geniculate awn, it is nonetheless valuable to the oat breeder as a source of resistance to crown rust and smut.—H. C. MURPHY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station.

A Method for Inoculating Wheat and Barley with Loose Smuts.[1]—Adequate testing of the reactions of varieties and selections of wheat and barley to loose smuts, as well as an extensive study of physiologic specialization in the smuts themselves, depends largely upon the use of a rapid and effective method of inoculation. An apparatus has been devised that subjects the wheat or barley head to a partial vacuum while completely submerged in an aqueous spore suspension. In this way as many as 30 heads per hour can be inoculated.

[1] Paper No. 1378 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

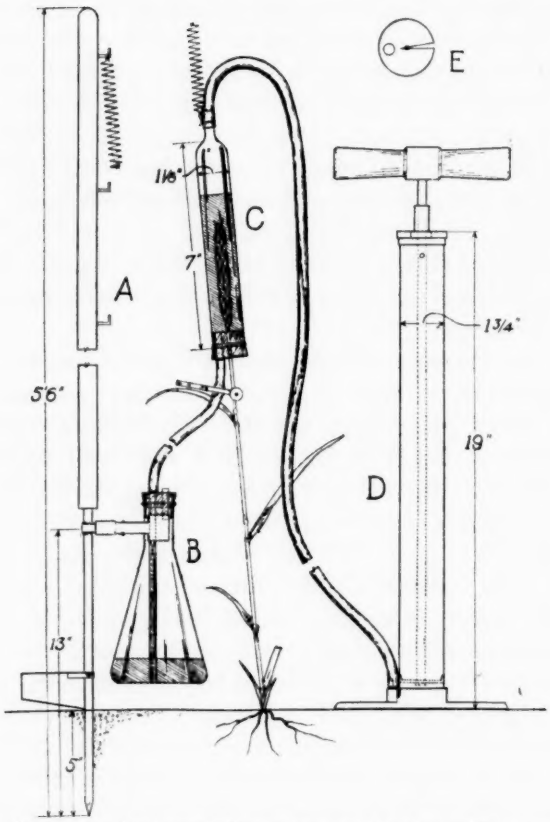


FIG. 1. Apparatus for inoculating wheat and barley with loose smuts.

The apparatus (Fig. 1) comprises a support (A); a flask for inoculum (B); an inoculating chamber (C); and a hand-operated vacuum pump (D). The lower portion of the support rod is made of 5/16 in. iron rod to which are welded an iron step to aid in pushing the rod into the ground and an old apparatus clamp to support the inoculum flask. The upper portion is best made of bamboo or light wood, equipped with a number of light, spring-wire hooks. The inoculating chamber can be made from a large test tube, or a long filter tube. The lower end (larger end) is fitted with a very soft, split rubber stopper (section at E, figure 1) and is connected through a length of rubber tube and a Mohr pinch cock with the inoculum flask. Four or five feet of thick-wall, small-bore tubing connect the top of the chamber with the vacuum pump. The inoculating chamber with stopper and tubes is hung from the support rod by a weak spring, such as may be taken from a small curtain roller. A large automobile pump with an inverted plunger leather makes a satisfactory vacuum pump. A $\frac{1}{8}$-in. vent hole is bored

through each side of the cylinder of the pump at a point just below the upper limit of operation of the plunger, so that normal pressure is suddenly restored when the plunger is raised above it. It is important to keep the internal volume not filled by liquid as small as possible in order to create the greatest possible vacuum with each stroke of the pump.

The procedure for inoculation is simple. Fill the inoculum flask (B) with a suspension of spores, made up in the proportion of about two medium-size smutted heads in 100 cc. of water. Push the support rod (A) into the ground near the plant to be inoculated and hang the spring and inoculating chamber on one of the hooks so that a slight extension is necessary to accommodate the chamber to the height of the plant. Spread the split rubber stopper and place it around the stem just below the head; then insert head and stopper into the inoculating chamber. With barley enclose the leaf sheath and the stem, since the stem alone is too weak to survive the treatment. Open the pinch cock between B and C and draw the inoculum up into C by a gentle stroke of the pump. When the head is covered, but the chamber not more than ¾ full, close the pinch cock and operate the pump vigorously. Four to six strokes will suffice for wheat, but barley may require 10 to 15 or even more.

The partial vacuum expands the air in the florets, and the returning pressure replaces it with the liquid inoculum. Some air will leak through and around the stem, but if the pump is worked rapidly this offers no serious difficulty. When inoculation is complete, the excess inoculum may be returned to the flask by opening the pinch cock after raising the pump plunger above the vent holes. In this way the liquid may be used repeatedly, and 300 cc. will be found sufficient to inoculate 60 to 80 heads.

Results from inoculations made in 1933 and preliminary results from inoculations made in 1934 indicate that the method is highly successful for

TABLE 1.—*Loose-smut infection in 1933 resulting from vacuum inoculation of Reward wheat*

Source of inoculum		Total plants	Plants smutted	
Place	Variety		Number	Percentage
Minnesota	Marquis	22	21	95
Minnesota	Reward	21	19	90
Minnesota	Minturki	25	24	96
Oklahoma	Unknown	24	24	100
South Dakota	Marquis	25	24	96
Texas	Durum (variety unknown)	23	0	0
Iowa	Winter wheat (variety unkown)	84	0	0
Check	Uninoculated	26	0	0

loose smut of wheat and somewhat less so for loose smut of barley. The results of inoculations on Reward wheat in 1933 are given in table 1. These inoculations resulted either in no infection or in a very high degree of infection, indicating that the variation was due to differences in strains of smut used rather than to inconsistencies in the method. Eight different collections of barley loose smut were inoculated separately into 2 to 7 varieties of barley in 1933. Infection in the resulting plants ranged from 2 to 76 per cent with an average of 26.4 per cent.

Inoculation somewhat reduces the number of seeds that develop per head. For this reason, only the most vigorous heads should be selected for treatment. The most favorable stage of development, both for set of seed and for infection, seems to be just after anthesis in most of the florets and before the ovaries have more than doubled their original size.—M. B. MOORE, University Farm, St. Paul, Minnesota.

Crown Gall on Araucaria Bidwillii.—Several of the conifers (*Juniperus Sabina* L.,[1] *Cupressus arizonica* Green,[2] *Sequoia gigantea* (Lindl.) Dee,[3] and *S. sempervirens* (Lamb) Endl.[3]) have been reported as susceptible to crown gall caused by *Pseudomonas tumefaciens*. *Araucaria Bidwillii* is another conifer that has been artificially inoculated with *Ps. tumefaciens* from peach. *A. Bidwillii* is indigenous to Australia, but is popular in California and Florida as a park and lawn tree.

The artificial aerial galls on *Araucaria Bidwillii* were 5–30 mm. in diameter and nearly spherical. The surface of the galls (Fig. 1, C) was almost smooth, but sometimes was characteristically sculptured by small, regular, angular, brownish areas, probably caused by unequal growth and

TABLE 1.—*Results from five-puncture inoculations with Pseudomonas tumefaciens on Araucaria Bidwillii in 1935*

Date of inoculation	Number of galls	Diameter in millimeters
January 3	2	5–25
February 23	2	10–30
April 6	3	5–20
April 16	1	5
May 18	0	0
May 24	0	0
July 5	0	0

[1] Savin (Juniperus Sabina). U. S. Dept. Agr. Bur. Plant Indus., Plant Dis. Rptr. Sup. 81: 133. 1931. (Mimeog.)

[2] Brown, J. G., and M. M. Evans. Crown gall on a conifer. Phytopath. 23: 97–101. 1933.

[3] Smith, C. O. Crown gall on the Sequoia. Phytopath. 25: 439–440. 1935.

tension in the tissue. Inoculations on a rapidly elongating internode gave negative results, the opposite of what was to be expected, and another test should be made. The largest galls were produced on trunk tissue (more than one-year-old) of a small tree growing in the lath house (Fig. 1, A). Galls also were made (inoculation of April 6, 1935) on last season's growth

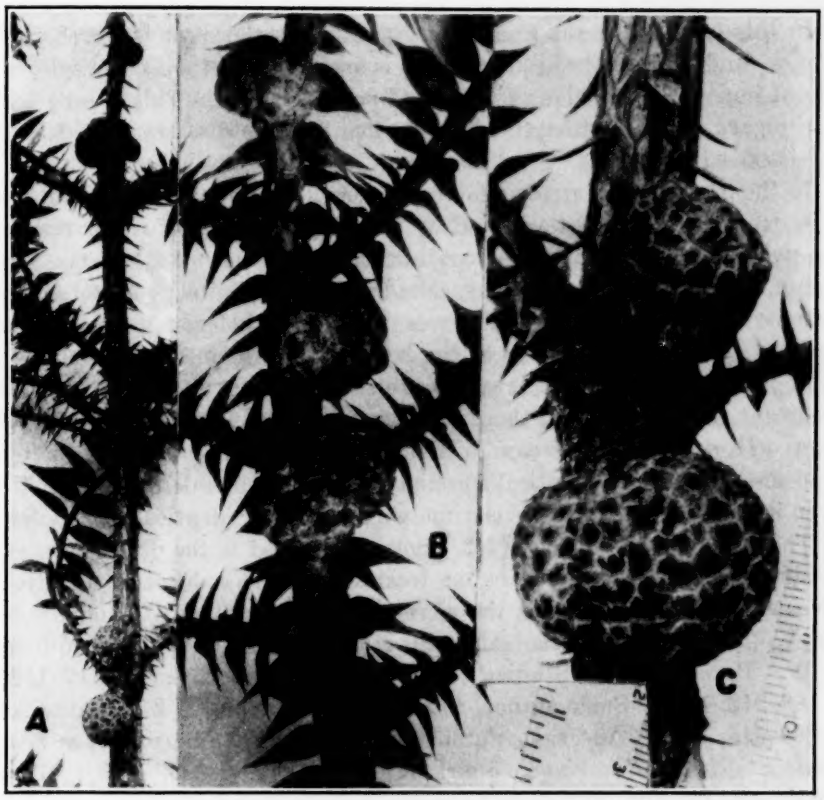


FIG. 1. Artificial inoculation on *Araucaria Bidwillii*. *A*. Galls (much reduced) of two different inoculations on a small tree growing in the lath house: at top, three galls developing from inoculation of January 3, 1935; at bottom, two galls resulting from the inoculation of April 6, 1935. *B*. Galls on a small branch of a street tree inoculated February 23, 1935. *C*. Two galls that were illustrated below in *A*. These are photographed slightly enlarged and show the characteristic breaking up of the surface.

of a lawn tree (Fig. 1, B). The tabulated results suggest that *A. Bidwillii* is fairly susceptible to crown gall and that the inoculations made in January to April gave positive results, while those made later were negative. The causal organism was reisolated from the galls made on April 6.—CLAYTON O. SMITH, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

BOOK REVIEW

Wollenweber, H. W., and O. A. Reinking. *Die Fusarien—ihre Beschreibung, Schadwirkung und Bekämpfung.* VIII. 355. 95 figs. Paul Parey, Berlin, 1935.

In this book the genus Fusarium is treated in the most comprehensive manner, and thus, for the first time, is brought together essentially all data of importance previously published on the subject. This is especially so in regard to description and classification and the diseases caused by or associated with Fusaria.

In the preface and introduction are given the high points of the subject, of its treatment in this work, and of the most promising further studies. The principle adopted as to the continuation of the use of the name "Fusarium," even after many of the species have been definitely connected with their perfect stages, is of special interest to plant pathologists. The authors consider such use of the name of the imperfect fungi justified because for many of the fungi the perfect stages are still unknown and, therefore, it is important that these fungi should be accurately known in their imperfect stages. However, in every case in which an Ascomycete is definitely known to possess a similar or identical conidial stage, a detailed description of it is given immediately after the Fusarium with similar or identical morphology.

The first part of the book (141 pages) is devoted to the description and classification of Fusaria. Here the treatment of the subject is essentially the same as that presented in the more recent publications of Wollenweber and Wollnweber in co-authorship with other workers. (See Wollenweber, H. W. Pyrenomyceten-Studien I and II. Angew. Bot. 6: 300–313, 1924; 8: 168–212, 1926. Same author, *Fusarium-Monographie.* Z. f. Parasitkde 3: 269–516, 1931. And same author with others, *Fundamentals for Taxonomic Studies of Fusarium.* Jour. Agr. Res. 30: 833–843, 1925). There is only one material deviation from the previously adopted nomenclature in the literature just cited, namely, that some species of Fusarium, previously described as "forms," are now given varietal names, when they are known to cause specific plant wilt. In this part of the text a general discussion of the genus Fusarium, and other genera closely related to it or identical with it, is followed by a list of all Fusaria now recognized, with corresponding Ascomycetes, the latter belonging to the following genera: Nectria, Calonectria, Gibberella, and Hypomyces. Then is given a list of the sections, 16 in all, with the Ascomycetes and the Fusaria belonging to each section; and this is followed by a classification key for the sections. Finally, each section is treated separately, with classification keys for all Fusaria known in each of the sections, and after each of the keys are

described all of the fungi, first under their Fusarium name and then under the name of the Ascomycete connected with it, whenever the latter is known. In the systematic portion of the book are described 65 species, 55 varieties, and 22 forms. All the numerous other names previously given are reduced to synonyms.

The larger part of the text, from page 142 to 302, is devoted to the plant diseases caused by or associated with the Fusaria, and their control. The diseases of feed and food crops are presented under the generic names of the hosts and the entomophthorous Fusaria under Citrus, where these fungi are of greater economic importance. Here, as in the first part, the subject is covered in a thorough manner, apparently without the omission of anything of importance.

Pages 302 to 316 are devoted to the Fusaria occurring on other fungi and on living animals, on raw materials and products of plants and animals, and also in soil and water. The next 22 pages present a complete list of the Fusarium synonyms and homonyms.

The entire presentation of the subject is excellent, and the material is most conveniently arranged and well illustrated. The book will be extremely helpful to plant pathologists and mycologists interested in any way in this numerous and important group of fungi.—C. D. SHERBAKOFF, University of Tennessee, Knoxville, Tenn.

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